Archives of Pathology

VOLUME 45 MAY 1948 NUMBER 5

COPYRIGHT, 1948, BY THE AMERICAN MEDICAL ASSOCIATION

A QUANTITATIVE HYPOTHERMAL METHOD FOR THE PRODUCTION OF LOCAL INJURY OF TISSUE

GEORGE M. HASS, M.D.
AND
C. BRUCE TAYLOR, M.D.
CHICAGO

CERTAIN STUDIES of local or systemic effects of injury of tissue require methods by which an injury of specific dimensions can be reproduced quantitatively in one or more locations in successive experimental animals. Methods such as those which employ mechanical trauma, surgical excision, arterial ligation, irradiation or cauterization have often been used. Each has advantages and disadvantages depending on the requirements of the experiment.

The purpose of this report is to describe an additional procedure, a hypothermal method, which has some advantages over other methods in special types of experiments.

APPARATUS

The apparatus to be described produces local injury of tissue by creating a negative thermal gradient from tissue to a contiguous instrument cooled by expanding carbon dioxide.

The apparatus consists of two assemblies. One assembly is concerned with the supply of carbon dioxide. The other is the hypothermal instrument by which the dimensions of lesions are controlled.

The supply of carbon dioxide is maintained in a standard commercial steel cylinder fitted with a pressure gage. The pressure in the cylinder is kept at 800 to 1,100 pounds per square inch. Carbon dioxide is conducted from the cylinder through flexible metal tubing to a needle valve which delivers a maximum flow when the manual control is turned through an angle of 45 degrees. Distal to the needle valve the metal conducting tube terminates in one of the two parts of a Luer-Lok joint. The hypothermal instrument, through the second part of the Luer-Lok joint, is attached to this terminal fitting.

The parts of the hypothermal instrument prior to assembly are shown in figure 1. All parts except the needle are made of brass. There are three essential units, namely, a needle, a hypothermal plate and the cylindric body of the instrument. The cylindric body of the instrument, as shown in figure 1, consists of three concentric cylinders open at one end and sealed together at the other. The internal cylinder is 75 mm. in length and 15 mm. in diameter. The wall is 4 mm. in thickness. The proximal 10 mm. of the wall is threaded internally to accept the screw into which the long needle is sealed. The

This research was aided by a grant from the Otho S. A. Sprague Memorial Institute Fund.

From the Rush Department of Pathology, Presbyterian Hospital, in affiliation with the Department of Pathology, University of Illinois College of Medicine.

remainder of the bore of the cylinder is tubular and, as shown in figure 1*B*, it opens distally in the center of a hemispheric chamber. The middle cylinder is 80 mm. in length and 22 mm. in diameter. Its wall is 1 mm. in thickness. The space between the inner surface of the wall of this cylinder and the external surface of the wall of the

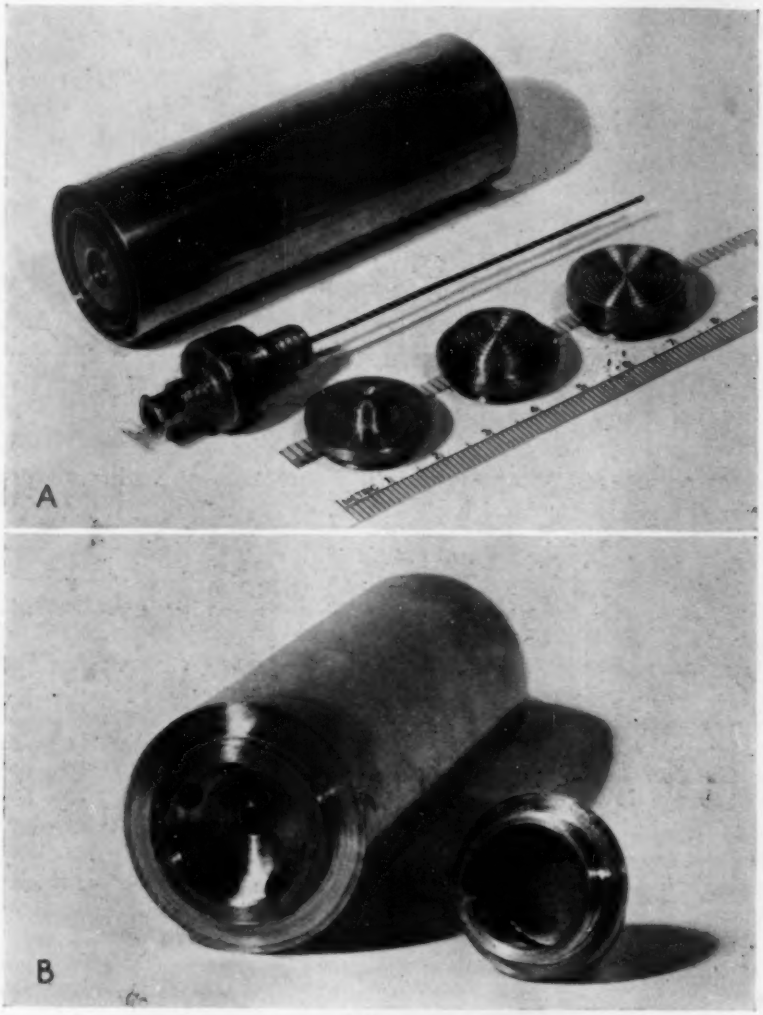

Fig. 1.—*A*, parts of the hypothermal instrument, prior to assembly.
B, interior of the expansion chamber of the hypothermal instrument and the inner surface of the largest of the three interchangeable hypothermal plates shown in *A*.

internal cylinder is 3 mm. in breadth. This space is open proximally, and communicates distally through eight holes with the hemispheric chamber (fig. 1*B*). The external cylinder is 80 mm. in length and 29 mm. in diameter. Its wall is 1 mm. in thickness. The space between the inner surface of the wall of this cylinder and the external surface

of the wall of the middle cylinder is 2 mm. in breadth. The space is open proximally but closed distally.

The needle, shown in figure 1*A*, is a 19 gage spinal needle, 120 mm. in length. Just distal to the Luer-Lok fitting, the shaft of the needle is soldered into the axis of a hollow brass block which is threaded distally to fit into the proximal end of the central cylinder of the main body of the instrument. When the brass block is screwed into the

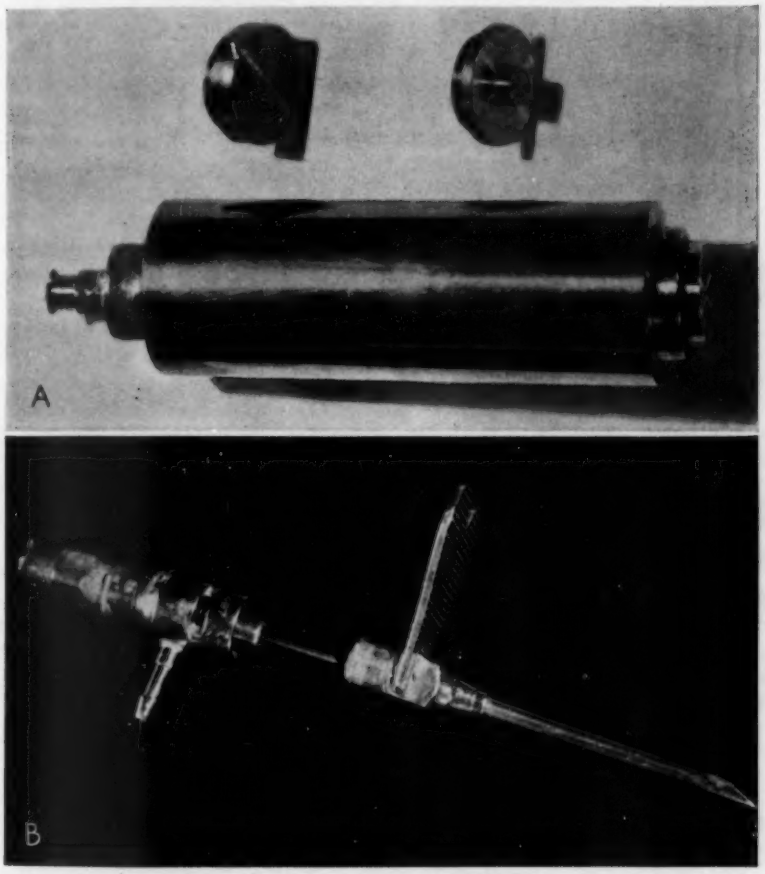

Fig. 2.—*A*, profile view of the assembled hypothermal instrument and two accessory hypothermal plates.
B, partly assembled hypothermal needle.

central cylinder, the tip of the needle projects into the hemispheric chamber at the distal end of the instrument as shown in figure 1*B*.

The third essential unit of the instrument is a hypothermal plate. The external surfaces of three plates of different dimensions are shown in figure 1*A*. The internal surface of the largest plate is shown in figure 1*B*. Each plate is machined out of solid brass. Proximally, each is threaded externally to fit a common set of threads at the circumference of the distal part of the hemispheric chamber shown in figure 1*B*. Each

plate is of the same height (9 mm.) and breadth (20 mm.). Each is hollow beneath the concentrically grooved surface, so that the thickness of the grooved part of each plate is uniform (0.5 mm.). The only essential difference between the plates is the diameter of the grooved surfaces. The surfaces shown here are 6, 15 and 20 mm. in diameter. When the plate is screwed in place, the tip of the needle lies 1 to 2 mm. proximal to the center of the internal surface of the grooved plate.

When the instrument is assembled, as shown in figure 2*A*, the carbon dioxide enters the needle through the union of the male and female components of the Luer-Lok joint distal to the needle valve which controls the flow of gas. The gas then traverses the needle, expands opposite the center of the inner surface of the grooved plate, and abstracts heat from the plate. It then further expands into the hemispheric chamber (now closed distally by the metal plate), leaves the chamber through the eight holes shown in figure 1*B* and passes back through the main body of the instrument in the space between the internal and middle cylinders to escape into the air of the room.

When the instrument is in operation, the center of the grooved plate is the point of lowest external surface temperature. Practically, the thermal gradient from the center to the margin of the grooved plate is slight. It appears that thermal flow from tissue in contact with the grooved hypothermal plate is nearly equal along any line perpendicular to the face of the plate.

Another hypothermal instrument shown in figure 2*B* has been designed for insertion into tissues. This is made of two needles. The external needle is a 15 gage intravenous needle with a Luer-Lok type of fitting and side-arm. The distal end of the bore of the needle is closed by a thin plate of solder. The internal needle is 19 gage with a Luer-Lok fitting. The shaft of this needle is soldered into the proximal fitting of the external needle so that the open tip of the internal needle is 1 to 2 mm. proximal to the closed tip of the external needle. The over-all length of the instrument is 85 mm. The length of the shaft of the external needle is 35 mm. This is the depth to which the instrument may be inserted into tissue.

When this instrument is in operation, it receives its supply of carbon dioxide through the Luer-Lok fitting of the internal needle. The gas passes down the bore of the internal needle and expands at the tip of the needle into the chamber between the external surface of the inner needle and the inner surface of the external needle. The rapidly expanding gas cools the external needle and then escapes through the sidearm into the air of the room.

GENERAL USE OF HYPOTHERMAL INSTRUMENTS

The maximal effect of the instrument with the cooling plate is exerted in a direction perpendicular to the surface of application of the plate. The surface of the tissue or organ is exposed, surgically if necessary. The cooling plate of desired surface area is screwed into the instrument. The surface of the cooling plate is immersed in a 10 per cent aqueous solution of gelatin at 50 C. The plate is then placed firmly against the tissue. Excess gelatin is wiped away, leaving a liquid film between the plate and the tissue. Carbon dioxide at 800 to 1,100 pounds (363.2 to 499.4 Kg.) per square inch (6.45 sq. cm.) is then jetted periodically against the inner surface of the plate. The duration of each jet and the interval between jets are timed with a metronome at one and two-tenths seconds. When a lesion of desired volume has been produced, the instrument and the tissue are allowed to gain heat from the environment. Within five minutes the tissue spontaneously separates from the cooling plate.

The hypothermal needle is most useful when deep cylindric lesions of small diameters are desired. The needle is inserted to the required depth. The flow of carbon dioxide is then controlled in the same way as described in the preceding paragraph.

After the lesion has been produced, the flow of gas is shut off. Within a short time the needle has warmed sufficiently so that it may be withdrawn from the tissue.

SPECIFIC USE OF HYPOTHERMAL INSTRUMENTS

Production of "Closed" Craniocerebral Lesions.—Experiments were done on the skulls and brains of albino rabbits 3 to 6 months of age and weighing 4 to 6 pounds (about 2 to 3 Kg.). After induction of anesthesia with ether, a midline incision about 1 inch (2.5 cm.) long was made in the scalp over the superior longitudinal synostosis. The scalp was then reflected so as to expose the superior surface of the frontal and parietal bones. The cooling plate was placed firmly against the skull to the right or the left of the midline over the part of the cerebrum in which the injury was to be produced. The carbon dioxide was then turned on. Within a few seconds the hypothermal plate was securely frozen to the skull. With continued extraction of heat from the tissue, the cylindric zone of the injury progressively deepened involving in succession the skull, the meninges, the cerebral cortex, the subcortical white matter and finally the wall of the lateral ventricle. When a lesion of desired depth had been produced, the carbon dioxide was shut off. Environmental temperature soon warmed the hypothermal plate so that it spontaneously separated from the periosteum of the external table of the skull.

Most experiments were concerned with the controlled production of lesions of different dimensions. As a rule, only one lesion was produced in the brain of each animal. At times, however, two or more lesions were made in different locations in the brain at intervals of time, varying from a few minutes to ninety-six hours. When the interval was more than a few minutes, the scalp was sutured and the animal allowed to recover from the anesthesia before another lesion was produced.

Animals with single or multiple cerebral lesions either died within a few hours or survived, later to be killed at appropriate intervals up to six weeks. Brains were removed and fixed in solution of formaldehyde U.S.P. The volume of each brain was then determined by fluid displacement. The volume of each lesion was calculated from measurements. Blocks of tissue from each lesion and the overlying calvarium were then prepared for microscopic study.

Production of Cardiac Lesions.—The hypothermal plate and needle have been used to produce cardiac lesions in dogs. Tidal intratracheal pressure anesthesia was used. The heart was exposed through a parasternal surgical approach. The parietal pericardium was incised. The hypothermal plate was pressed against the epicardium overlying the region to be inactivated. Care was taken to avoid large coronary arteries. The carbon dioxide was turned on, and shortly thereafter the hypothermal plate was frozen securely in place. With continued flow of the gas, the lesion progressively deepened, involving in succession the epicardium, the myocardium and finally the endocardium. When a lesion of desired dimensions had been produced, the carbon dioxide was shut off. Warm saline solution was then poured over the hypothermal instrument until it spontaneously separated from the epicardium. The parietal layer of the pericardium and the tissues of the thoracic wall were then closed surgically.

Numerous lesions of different dimensions were produced in many locations in the walls of auricles and ventricles. Surviving animals were killed at appropriate intervals up to six weeks. Each heart was fixed in solution of formaldehyde U.S.P. The volume of each lesion was calculated from measurements. Blocks of each lesion were then prepared for microscopic study.

The hypothermal needle was used to produce discrete lesions of the interventricular septum of the dog's heart. The surgical approach to the heart was similar to that just described. The needle was then pushed through the wall of the right ventricle into the

interventricular septum. Carbon dioxide was turned on until a lesion of desired size was produced. Dogs were then killed at appropriate intervals. Septal lesions of various ages, dimensions and locations thus became available for gross and microscopic study.

Production of Hepatic and Renal Lesions.—Lesions of the livers and the kidneys of rabbits have been produced by use of the hypothermal plate. The organs were exposed surgically with the animals under ether anesthesia. The hypothermal plate was placed firmly against the capsule of the liver or that of the kidney. The technic of this use of the instrument was essentially the same as that employed in producing lesions of the heart. Animals were killed at appropriate intervals up to five weeks. The tissues were fixed in solution of formaldehyde U.S.P. Lesions of different ages, dimensions and subcapsular locations were then studied by gross and microscopic methods.

DETERMINATION OF VOLUMES OF LESIONS

The volume of each acute lesion was determined in the following way: The organ with the lesion was fixed in solution of formaldehyde U.S.P. The volume of the organ was determined by its capacity to displace fluid. The surface area of the exposed base of the cylindric lesion was then reproduced accurately on graph paper having transverse and vertical lines at intervals of 1 mm. The surface area was then calculated by counting the number of squares on the graph included within the perimeter of the outline of the lesion. Sections were then cut through the diameter of the exposed surface area in a direction perpendicular to the surface. The depth of the lesion was then measured in millimeters. The product of the surface area and the depth was a fair approximation of the volume. The ratio between the volume of the lesion and the volume of the organ gave other useful quantitative data.

The volumes of chronic and healed lesions were estimated by similar measurements. These estimates were less reliable because of the contraction and irregularity of volumes of injured tissue as resolution and repair proceeded.

GENERAL RESULTS

The usual acute lesion produced by the hypothermal plate was cylindric. The area which had been in contact with the cooling plate was circular, with a diameter slightly greater than that of the plate. Its margins were sharp. Its contour was convex as the result of edema and interstitial hemorrhage. The opposite base of the cylindric lesion represented the limit of the lesion in depth. It was circular with a diameter slightly less than that of the hypothermal plate. It was slightly convex, owing to rules governing the flow of heat. At all levels between the two bases of each lesion, the line of demarcation between normal and inactivated tissue was sharp.

The depth of the lesion depended principally on the type of tissue and the duration of cooling. If the duration of cooling was held constant, the degree of vascularity of the tissue was the principal variable which determined depths of lesions. In general, however, there was uniform inactivation of tissue to a depth of 4 to 6 mm. in one minute, 6 to 8 mm. in two minutes and 8 to 9 mm. in three minutes. With prolonged cooling, it was possible to produce lesions that were 12 to 13 mm. in depth.

The cylindric volume of tissue inactivated by the hypothermal needle was slightly greater in length than the part of the needle inserted

into the organ. The needle produced deep lesions having a more uniform diameter than those made by the hypothermal plate. As when the hypothermal plate was used, the principal variables which influenced the diameter were duration of cooling and vascularity of the tissue. In general, inactivation of tissue proceeded at equal rates radially from the needle as a central axis. The radius of inactivation was about 3 to 4 mm. in the first minute, 4 to 5 mm. by the end of the second minute and 5 to 6 mm. by the end of the third minute of cooling. The maximal diameter of a cylindric lesion was therefore about 12 to 14 mm. and the maximum depth was about 35 mm.

Lesions within the range of effectiveness of the instruments were reproduced in successive animals with considerable accuracy. The accuracy by which location could be reproduced depended principally on mechanical placement of the instrument. Quantitative reproducibility of the lesion depended principally on adherence to standard technic in controlling the flow of carbon dioxide at adequate pressure. From a qualitative standpoint, reproducibility of the degree of inactivation was a natural result of the standardized method by which the tissue was damaged.

SPECIFIC RESULTS IN VARIOUS ORGANS

"Closed" Craniocerebral Lesions.—In the production of "closed" craniocerebral lesions the use of the hypothermal plate gave the following results: During the first few seconds of operation the hypothermal plate was rigidly frozen to the periosteum of the external table of the calvarium. Within thirty to sixty seconds the advancing plane of progressive inactivation of tissue extended through the calvarium, the dura and the leptomeninges. After sixty to ninety seconds the deep plane of demarcation between normal and inactivated tissue was in the cerebral cortex or the underlying white matter. After ninety to one hundred and eighty seconds the lesion extended to some level between the cortex and the wall of the lateral ventricle. If the lesions were not too deep or too great in volume, the animals made a normal recovery from the anesthesia and thereafter exhibited no signs or symptoms. If the depth and volume of lesions approached the lethal range, the animals showed an increasing tendency toward delayed recovery of consciousness and secondary lapse into unconsciousness, with death in a few hours. Paralytic manifestations were never encountered in conscious animals, irrespective of the magnitude of lesions within the parietal, occipital and frontal lobes subjected to study.

It was apparent from a study of lesions made immediately after the injury had been produced that some tissues were damaged more than others. There was little or no gross or microscopic change in the periosteum, the bone or the dura. There was pericapillary hemorrhage

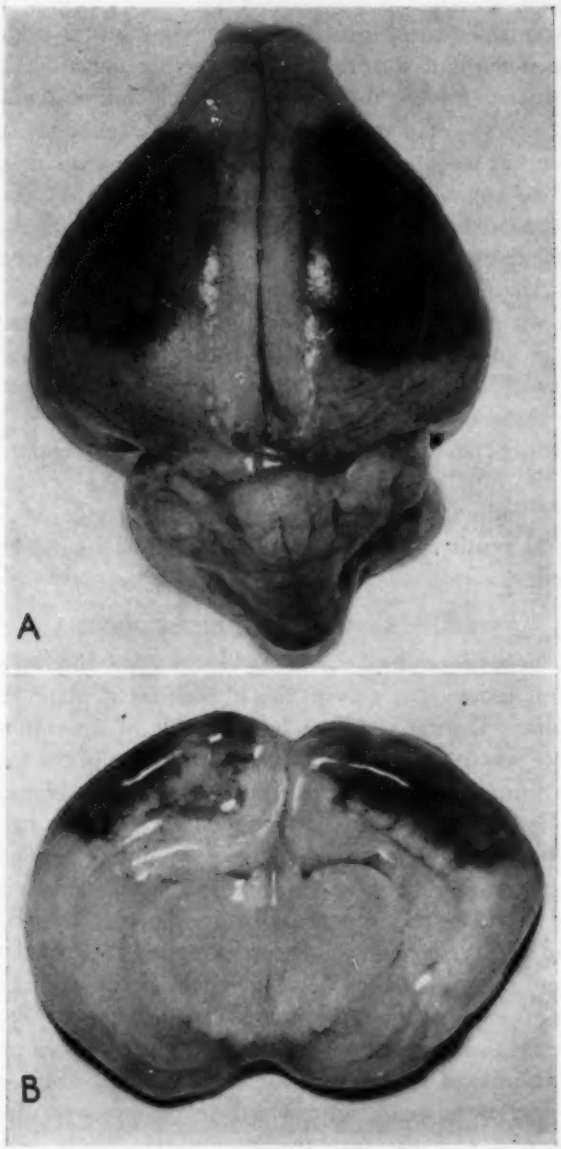

Fig. 3.—*A*, usual gross external appearance of acute symmetric bilateral hypothermal lesions of the brain of a rabbit. The duration of operation of the instrument was two minutes over each parietal lobe. The animal died four hours after production of the lesions. Note the similarity of the lesions, their sharp contour and the mild hemorrhage, sharply restricted to the site of each lesion.

B, appearance in cross section of acute symmetric bilateral hypothermal lesions of the brain of a rabbit. The duration of operation of the instrument over each parietal lobe was two minutes. The animal died six hours after production of the lesions. Note the sharply defined margins and bases of the lesions. Edema and minute perivascular hemorrhages, restricted to the sites of the lesions, are conspicuous.

in the leptomeninges and the brain, but in all instances the hemorrhage was greatest in the substance of the brain and was invariably restricted to the site of the lesion (fig. 3). No oozing into the subarachnoid space beyond the sharp limits of the lesion was encountered. Hemostasis seemed spontaneous and efficient.

The study of lesions a few hours after production disclosed no gross changes other than mild edema in the periosteum, the bone or the dura. Microscopic examination showed a few agglutination thrombi and infiltrating leukocytes, but the inflammatory reaction was mild. The most severe effects were in the bone marrow, where disintegration of hemopoietic tissue was conspicuous. Vasodilatation, edema, pericapillary hemorrhage and degeneration of nerve cells were conspicuous in the cerebral cortex (fig. 8*A*). Endothelial cells, fibrocytes and microglial cells often seemed structurally unimpaired. Leukocytes were few. Similar changes were found in the subcortical and the paraventricular tissue.

The study of the evolution of the lesions indicated that over a period of several weeks no significant gross changes developed in the periosteum or the bone. There was no suppuration or sequestration. The tissues retained normal anatomic relationships and apparent continuity of structure. The early mild periosteal inflammation subsided without significant microscopic structural changes. There was evidence, microscopically, of a reorganization of bone matrix with marked delay in restitution of hemopoietic activity in the marrow. The dura showed local fibrosis with firm local adhesions to the pia-arachnoid at the site of the lesion. The pia-arachnoid was likewise increased in thickness because of the proliferation of fibrous tissue which bound it to the surface of the circumscribed lesion of the cerebral cortex. The lesions of the brain did not suppurate. The various components of the gray and white matter were gradually resorbed or removed by phagocytic cells as they disintegrated. As this process continued, the lesions became yellow and contracted. By the end of five weeks the large, edematous, purplish red acute lesions had been converted to small, puckered glial scars containing small cysts and numerous mononuclear macrophages laden with lipoid droplets.

Cardiac Lesions.—In the production of cardiac lesions the use of the hypothermal plate gave the following results: During the first few seconds of operation the grooved surface of the hypothermal plate became firmly frozen to the epicardium and subjacent contracting myocardium. Within thirty to sixty seconds the advancing plane of inactivation was deep in the myocardium. Within sixty seconds to three minutes, depending on the thickness of the myocardium, a cylindric volume of the musculature with epicardial and endocardial bases was

totally inactivated. Unless ventricular fibrillation developed during or immediately after the production of lesions, the dogs recovered uneventfully and thereafter exhibited no important signs or symptoms unless the volumes of lesions were excessive. In general, however, lesions of the ventricular walls, 20 mm. in diameter and involving the entire thickness of the myocardium, were well tolerated.

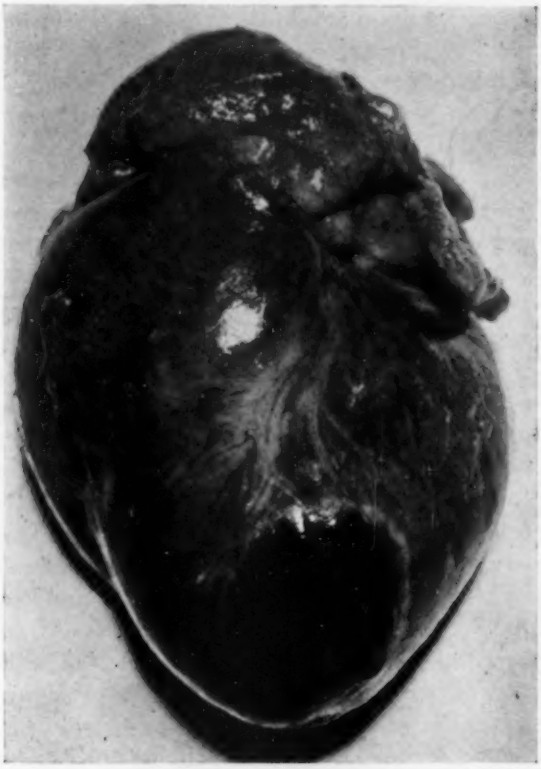

Fig. 4.—An acute hypothermal lesion of the apex of the left ventricle of a dog's heart. The duration of operation of the hypothermal instrument was two minutes. The lesion is two days old. The hemorrhage is perivascular, restricted to the zone of injury and distributed uniformly throughout the volume of inactivated muscle.

Immediately following the production of lesions, vasodilatation became conspicuous in the epicardium and the myocardium. There were widespread pericapillary hemorrhage and edema, sharply limited by the boundaries of the lesion. Spontaneous hemostasis occurred promptly (figs. 4 and 5).

The study of the cardiac lesions, a few hours old, showed a slight increase of edema and a mild fibrinous exudate on the epicardium overlying the inactivated myocardium (fig. 8*B*). Microscopic examination showed a few agglutination thrombi in small blood vessels. The cells

of cardiac muscle showed numerous minor structural alterations similar to those found in acute human myocardial infarcts. There were few infiltrating leukocytes. Fibrocytes, endothelial cells and collagen were surprisingly well preserved. No thrombi were found adjacent to the endocardium, even when this structure was involved by the lesion.

The study of the evolution of the lesions showed progressive resorption of extravasated blood and degenerated cardiac muscle fibers. Fibroblasts and newly formed capillaries became conspicuous. Collagen

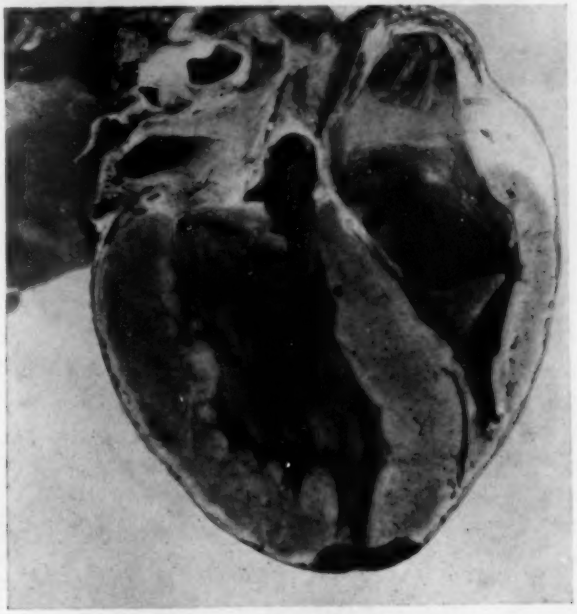

Fig. 5.—A coronal section through an acute hypothermal lesion of the apex of the left ventricle of a dog's heart. The duration of operation of the hypothermal instrument was ninety seconds. The lesion is two days old. Note the sharp transition between the lesion and the normal tissue at its margins and base. The dark color of the lesion is due to interstitial perivascular hemorrhage. There is homogeneous inactivation of all muscle within the boundaries of the lesion.

was gradually deposited. There was never any suppuration, rupture of the myocardial wall or significant aneurysmal dilatation. A thin, contracted, depressed cylindric scar, often bound to the parietal pericardium by fibrous adhesions, was the final result of healing. The principal difference at any stage between these lesions and human myocardial infarcts due to occlusion of coronary arteries was that the experimental lesions were always homogeneous and very sharply defined.

The quantitative technic of production of lesions of the interventricular septum by use of the hypothermal needle has not been fully developed. Lesions, however, which have been made in the septum do

not differ in any essential feature from those described in the external walls of the ventricles and auricles.

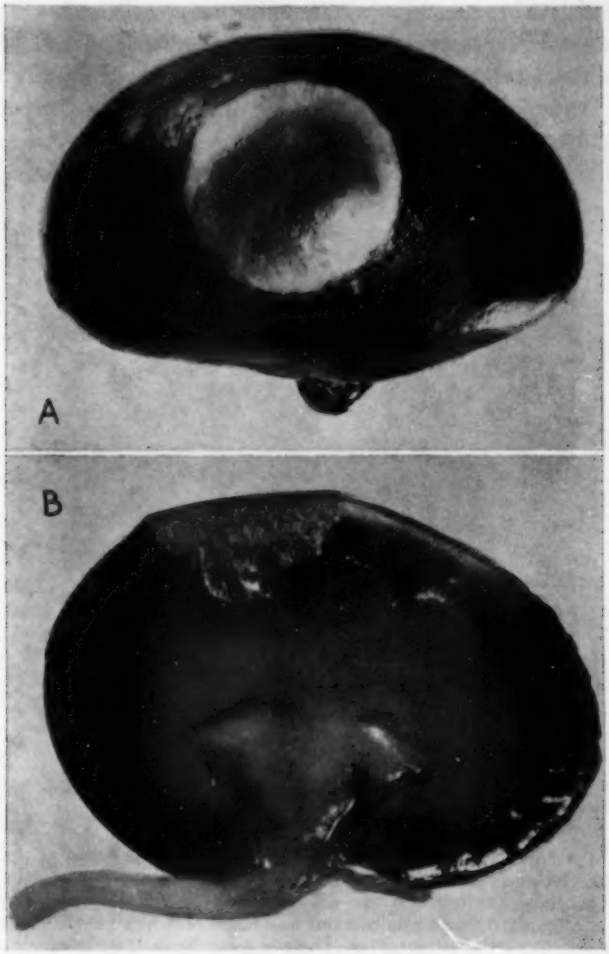

Fig. 6.—*A*, external appearance of an acute hypothermal lesion of the kidney of a rabbit. The duration of operation of the hypothermal instrument was two minutes. The lesion is four days old. Note the homogeneous, ischemic, sharply demarcated character of the lesion.

B, coronal section through the renal lesion illustrated in *A*. The margins of the lesion are sharp but are somewhat irregular at the base. Grossly, all renal lesions resembled ischemic infarcts. Hemorrhage was encountered only at the extreme periphery of a lesion.

Renal Lesions.—In the production of lesions of the kidneys of rabbits the use of the hypothermal plate gave the following results: Within the first few seconds the circular hypothermal plate was securely frozen to the capsule of the kidney. Within sixty seconds the advancing plane of inactivation had passed through the renal cortex. After one hundred and eighty seconds the plane of inactivation was

deep in the medulla. All animals recovered uneventfully and had thereafter no important signs or symptoms. Multiple bilateral lesions have not yet been thoroughly studied but single unilateral lesions seemed to be innocuous.

Immediately after the hypothermal plate was removed from the capsule of the kidney, a sharply defined circular grayish-white zone indicated the extent of the subcapsular lesion (fig. 6*A*). Sections perpendicular to the diameter of this zone showed that the gray discoloration extended, converging slightly, to the somewhat irregular deep base of the lesion in the cortex or the medulla (fig. 6*B*). The tissues within the volume of the lesion appeared ischemic. Vasodilatation and minimal interstitial hemorrhage, spontaneously controlled, appeared only at the zone of demarcation between normal and damaged tissue.

During the first few hours the only gross change in the appearance of the lesion was a mild, increasing edema. Microscopic study showed interstitial edema, agglutination thrombi in many vessels and autolytic changes in glomeruli and tubules. General outlines of all cellular structure persisted. Endothelium, fibrocytes and collagen showed the least changes. Leukocytic infiltration was mild and restricted to tissue at the margins of lesions.

During the succeeding few days the edema disappeared. Lesions contracted in size. The grayish white pallor of tissue was conspicuous. There was gross evidence of deposition of calcium. Microscopic study showed slow disintegration of cellular structure with little evidence of active vascularization or cicatrization of lesions (fig. 8*D*). The residual cytoplasm of many cells of the convoluted and collecting tubules was rather rapidly replaced by massive deposits of calcium.

By the end of the fourth or fifth week the healing by vascularization and fibrosis was still incomplete. Calcium was still being deposited. There was no evidence of suppuration or significant regeneration. Apparently, complete healing required a longer time than five weeks.

Hepatic Lesions.—All hepatic lesions of rabbits were produced by placing the hypothermal plate against the hepatic capsule. Animals with single lesions of dimensions within the limits of action of the instrument recovered promptly and had uneventful postoperative courses.

Within the first few seconds of operation of the instrument the hypothermal plate became firmly frozen to the hepatic capsule. Continued operation produced a gradually deepening lesion, which was 10 to 12 mm. in depth at the end of three minutes. On detachment of the instrument the capsule appeared unchanged. Beneath the portion of the capsule to which the plate had been applied, there was a sharply defined circular grayish white lesion with a hyperemic margin (fig. 7*A*). Incisions into the liver in a direction perpendicular to the subcapsular surface of the lesion showed a cylindric gray volume of inactivated

tissue (fig. 7*B*). The line of demarcation between the damaged tissue and adjacent normal tissue was sharp and characterized by local vasodilatation in normal tissue. Changes within the volume of inactivated tissue were homogeneous. There was no hemorrhage. Edema was mild.

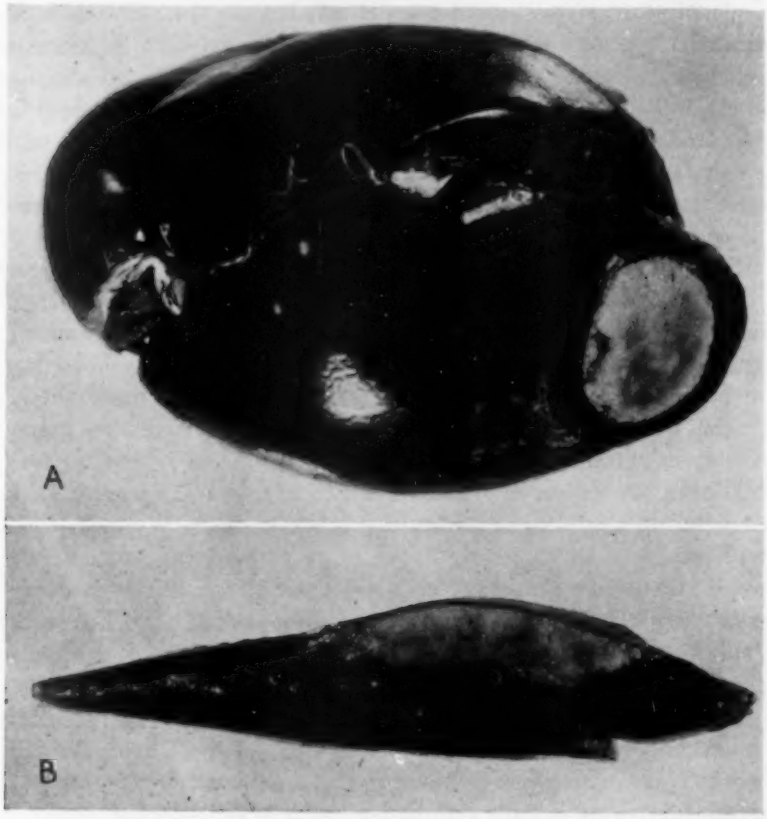

Fig. 7.—*A*, acute hypothermal lesion of the liver of a rabbit. The duration of operation of the hypothermal instrument was two minutes. The lesion is four days old. Note the homogenous, ischemic, circumscribed character of the lesion.

B, coronal section of the hepatic lesion illustrated in *A*. Note the pallor and absence of hemorrhage. Necrosis of hepatic cells is uniform throughout the lesion.

Gross and microscopic studies at successive stages in the healing of hepatic lesions led to the following conclusions: During the first few hours the principal findings were agglutination thrombi and coagulation necrosis of hepatic cells. During the succeeding days the coagulum became more homogeneous as outlines of nonviable hepatic cells and vascular sinusoids became less distinct (fig. 8*C*). However, anatomic relations still persisted, and the collagenous framework seemed to retain much of its normal structure. Proliferation of fibroblasts, formation of

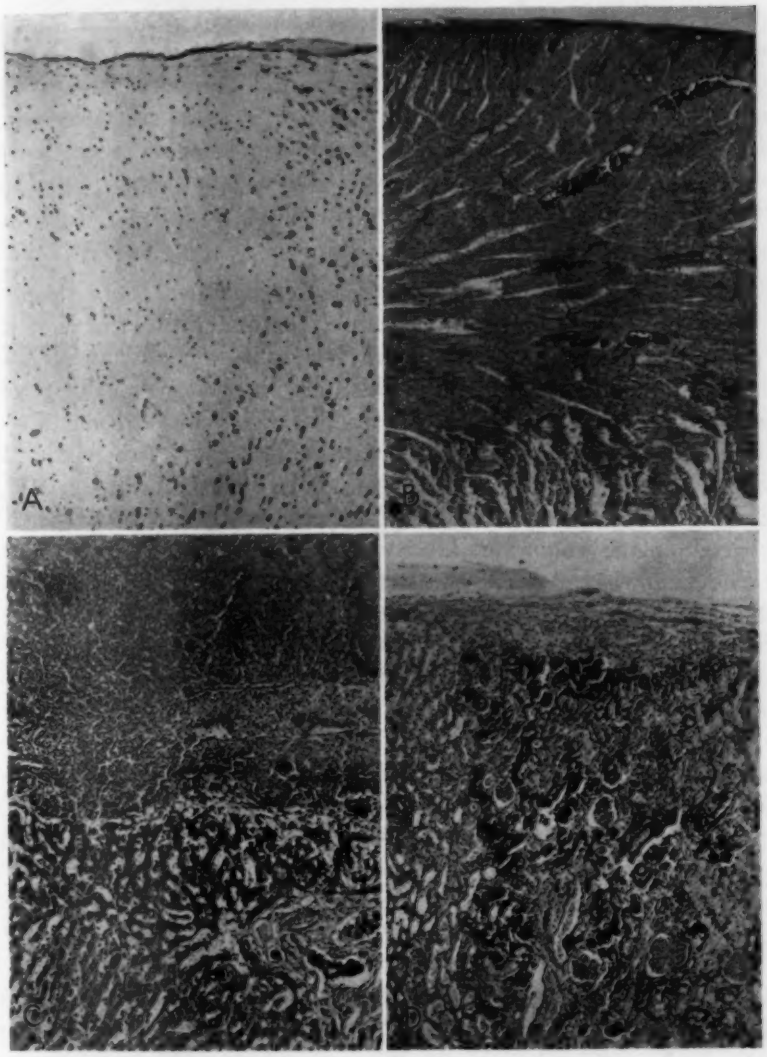

Fig. 8.—*A*, margin of one of the cortical lesions illustrated in figure 3*A*. Note the sharp line of demarcation between normal cortex and inactivated cortex. Only shrunken remnants of ganglion cells remain within the limits of the lesion. Inflammation is mild. Hemorrhage is not conspicuous.

B, full thickness of the lesion illustrated in figure 5.The epicardium and upper four fifths of the myocardium have been inactivated. The lower one fifth of the myocardium, shown here, is structurally normal. Continuity of cellular outlines is still retained within the volume of inactivated muscle.

C, base of the hepatic lesion illustrated in figure 7*B*. Note the coagulation necrosis with retention of vague outlines of lobular structure within the boundary of the lesion. There is a sharp transition from completely inactivated to normal tissue at the boundary.

D, capsular and subcapsular margin of the renal lesion illustrated in figure 6*B*. There is a sharp transition from normal cortex to inactivated cortex. Outlines of glomerular and tubular structure still persist within the lesion. The numerous dark masses which conform to the distribution of tubules are masses of calcium stained with hematoxylin.

collagen and regeneration of biliary ducts occurred at the margins of the lesion, but resolution and organization of the nonviable tissue within the lesion was a slow process. Leukocytic infiltration was never conspicuous. Suppuration did not occur. Calcium deposition, so characteristic in the healing of renal lesions, was insignificant or absent in the healing of hepatic lesions. Repair was still incomplete at the end of five weeks, the termination of the period of the study.

COMMENT

When it is desirable to inactivate a discrete, circumscribed volume of tissue while still retaining anatomic continuity of structure, the hypothermal technic may be useful. It has certain advantages over other methods that might be chosen. First, the volume can be inactivated without subsequent suppurative complications. Second, inactivation is achieved by a method which is essentially hemostatic. Although mild perivascular hemorrhage occurs during the production of lesions in some organs, the bleeding is temporary and spontaneously restricted to the volume of inactivated tissue. Third, the degree, the location and the quantity of inactivation may be controlled within defined limits. The degree of inactivation is controlled by action of a reproducible injurious thermal gradient in tissue. The location of inactivation is controlled by initial accurate placement of the instrument, which becomes firmly frozen to the area within a few seconds. Slipping or sliding of the instrument during the long periods necessary for producing maximal lesions does not occur. The quantity of inactivated tissue is controlled by standardization of the procedure with respect to the duration of the flow of carbon dioxide and the surface area of the hypothermal plate or needle in contact with the tissue. Reproduction of lesions in successive animals is fairly accurate with respect to area, depth and volume. For instance, the maximum deviation in a series of 100 closed craniocerebral lesions was plus or minus 10 per cent of the desired volume. Fourth, inactivation can be produced without disturbing the fibrous anatomic continuity of tissue. Fifth, healing proceeds slowly without serious local complications in any organ studied up to this time. Sixth, conditions of inactivation may be varied so that even though cells may be rendered nonviable, certain enzymatic systems and intercellular matrices may retain a degree of viability which can be determined.

The hypothermal instruments and methods have disadvantages which limit their usefulness in many types of experiments. There is a limit to the range at which the hypothermal plate or needle creates a lethal thermal flow out of adjacent tissue. This range is less than 14 mm. in a direction perpendicular to the surface of the hypothermal plate and about 6 mm. radially from the surface of the needle. A greater range is, of course, possible if materials capable of depressing tempera-

ture to a lower level than that obtained by expansion of carbon dioxide are used. A second disadvantage of the method is that inactivation is continuous, affecting all tissues from the surface of application of the instrument to the deepest limits of the lesion. There is no selective action on cells at a distance with perfect preservation of intermediate tissue. A final disadvantage is that healing of all lesions is slow.

Despite these disadvantages, hypothermal inactivation is at times more useful than surgical methods which depend on excision of tissue or ligation of blood vessels. Excision requires that anatomic continuity be interrupted. Hemorrhage is variable in quantity and seldom can be accurately controlled. Strictly quantitative studies of the dimensions of small lesions are difficult, if not impossible. Finally, excision of tissue defeats the objective of studies in which it is necessary that the inactivated tissue remain in its normal anatomic position. Inactivation of tissue by compression or ligation of arterial channels would seem to be at least as useful as the hypothermal method. Actually, attempts to produce lesions of the type described in this report by vascular ligation have failed. The variations of vascular distribution among animals and the inaccessibility of small nutrient vessels often offer insurmountable difficulties. Reproducible discrete topographic inactivation by vascular ligation can succeed only when the volume of tissue to be inactivated has a constant blood supply that can be ligated to achieve this purpose and no other.

Hypothermal methods have several advantages over hypothermal cauterization. The use of excessive heat leads to charring and sloughing of tissue, especially when deep thermal injury is desired. Furthermore, high temperatures have a destructive denaturing effect on many chemical compounds, especially proteins. These compounds, at least in vitro, are much more resistant to the effect of low temperatures. Finally, most biologic enzymatic systems are promptly and permanently inactivated by brief exposure to high temperatures. After exposure to low temperatures these same systems often remain unimpaired and display undiminished activity when the normal thermal environment is reestablished.

It may be useful at this time to describe briefly certain types of experiments which are now being done with hypothermal instruments. The mean lethal volume of "closed" cerebral injury in rabbits is being determined under conditions which eliminate mechanical trauma, concussion, uncontrolled hemorrhage, interruption of continuity of the cranial vault and infection as variables. The rate of onset and subsidence of cerebral edema is being determined under conditions closely simulating those of certain types of cerebral vascular accidents. The relative merits of methods designed to alleviate the undesirable effects

of increasing intracranial pressure are being evaluated, quantitatively. Other experiments are concerned with cerebral physiology, which is being studied by inactivation of large volumes of the cerebrum without introducing the undesirable complications which so often follow craniotomy and surgical decortication.

Hypothermal methods are being used in a topographic quantitative study of myocardial inactivation in dogs. The relationship of the volume and the location of inactivated cardiac muscle to electrocardiographic changes is being analyzed. Experiments are being done to determine the effects of gradual regional inactivation of other organs and tissues, with particular emphasis on bone, cartilage and the walls of blood vessels. It is hoped that, as these experiments proceed, the attention of surgeons will be drawn to the possibility that hypothermal instruments might be designed to simplify certain technical problems of human surgery.

SUMMARY

Local permanent inactivation of tissue can be produced rapidly and quantitatively by instruments cooled to a low temperature by expansion of carbon dioxide.

Two types of cooling elements have been used. One is flat and circular, so that when it is applied to the surface of an organ or tissue discrete cylindric lesions are produced. The diameters of lesions can be varied from 2 to 25 mm. by using cooling elements of comparable diameters. The depths of lesions can be varied from 1 to 13 mm. by varying the time during which the flat circular surface of the cooling element is in contact with the tissue. The second type of cooling element is a needle which can be inserted into tissue. Lesions produced by this instrument are cylindric. The diameters of lesions may be varied from 2 to 12 mm. and the depths from 2 to 35 mm.

The volumes of injured tissue are sharply defined and can be accurately measured. Lesions similar in quality, location and dimensions can be successively reproduced in the skull, the brain, the heart, the liver and the kidneys.

Necrosis of cells is uniform throughout the lesions, and there is sharp demarcation between nonviable and viable cells at the periphery. Intercellular matrices are surprisingly well preserved. Hemorrhage, if it occurs, is capillary in type, spontaneously controlled and restricted to the volume of injured tissue. Suppuration does not occur. Healing of lesions is slow but otherwise uncomplicated.

Organs and tissues can be topographically inactivated in a controlled quantitative manner which is not possible by use of methods such as hyperthermal cauterization, vascular ligation or surgical excision.

TESTICULAR TUMORS

I. Seminoma and Teratoma

ROBERT E. SCULLY, M.D.
AND
ASA R. PARHAM, M.D.
BOSTON

CONSIDERABLE CONFUSION exists in the medical literature on the subject of testicular tumors. This is due in part to the lack of a standard nomenclature and in part to the failure of numerous authors to emphasize the distinctive clinical and pathologic features of some of these tumors. The present study consists in a brief review of certain important contributions made to the literature and a clinicopathologic analysis of 37 cases which appeared in the files of the department of pathology of the Peter Bent Brigham Hospital from 1914 to 1947.[1] The study is divided into two parts. Part I is restricted to a discussion of the common testicular tumors, seminoma and teratoma; 33 cases are analyzed. Part II deals with interstitial cell tumors and a heterogeneous group of rare neoplasms; 4 cases are reported.

MATERIAL AND METHODS

The material consisted largely of surgically removed primary tumors and excised metastatic lesions or biopsy specimens of these. In addition, autopsy was performed in 9 cases. A minimum of 2 and usually 5 or more blocks were taken from each growth, fixed in Zenker's acetic acid solution and/or 4 per cent formaldehyde solution, embedded in paraffin, and stained with eosin—methylene blue and/or hematoxylin-eosin. Staining for connective tissue and fat was done when indicated. In 4 cases the microscopic sections of the primary tumor were prepared elsewhere, while in 4 cases no sections of the primary tumor were available for study (see tables 1 and 2).

GENERAL COMMENT

Tumors of the testicle constitute 0.5 to 2 per cent of all cancers in males.[2] They occur at all ages, the maximum incidence being between

---

From the Departments of Pathology of the Peter Bent Brigham Hospital and Harvard Medical School and the Department of Surgery of the Peter Bent Brigham Hospital.

1. Twenty-one of these cases have been discussed from clinical and pathologic points of view by W. C. Quinby (Rhode Island M. J. **21**:119, 1938) and J. E. Adams (J. Urol. **47**:491, 1942).

2. *(a)* Hinman, F.: The Principles and Practice of Urology, Philadelphia, W. B. Saunders Company, 1937. *(b)* Young, H. H.; Davis, D. M., and Johnson, F. P.: Young's Practice of Urology, ibid. 1926, vol. 1. *(c)* Nash, L. A., and Leddy, E. T.: Am. J Roentgenol. **50**:162, 1943.

20 and 50 years.[2e] Less than 3 per cent occur under the age of 15.[3] Approximately 1 in 8 is located in a cryptorchid organ.[2e] The right testis is involved slightly more frequently than the left, possibly owing to its higher incidence of cryptorchidism.[2e] Rarely a testicular tumor is bilateral.

A history of trauma has been reported in from 20 to 68 per cent of cases, but most authors have minimized its significance as an etiologic factor.[2e]

The most common presenting clinical manifestation is a testicular mass. Symptoms referable to metastases occasionally precede recognition of the primary tumor; it is quite rare, however, for the latter to be too small to be detected by palpation when the patient presents himself for the first time.[4]

Testicular tumors metastasize primarily by way of the lymphatic channels.[2e] These for the greater part drain directly into the abdominoaortic nodes, which extend from the bifurcation of the aorta to the level of the renal veins. There is widespread anastomosis between both sides. The abdominoaortic nodes drain into the lumbar trunks, which ascend through the aortic hiatus of the diaphragm to the thoracic duct. According to certain authors,[5] there are often one or more channels from the testis which empty directly into the mediastinal nodes. Commonly, enlargements of the retroperitoneal lymph nodes give rise to the earliest and most striking clinical manifestations of lymphatic dissemination; considerably less frequent is prominent involvement of the mediastinal or left supraclavicular nodes. Enlargement of the inguinal nodes indicates invasion of the parietal tunics and the scrotum. Blood stream metastases are likewise common; they are most frequent in the lungs but may be widespread.

Patients with testicular tumors usually excrete measurable quantities of gonadotropins in the urine. Two distinct types have been identified and may be distinguished by numerous biologic tests, the most practical of which utilizes intact infantile female rats or mice.[6]

The first type of gonadotropin is biologically similar to anterior pituitary gonadotropin. Small quantities are normally present in the blood and the urine; increased amounts have been reported in castrates, in women past the menopause, and in elderly men. When this type is injected into infantile rats and mice, it stimulates maturation of numerous ovarian follicles and, in larger doses, causes luteinization of the

---

3. Gilbert, J., cited by Matassarin, F. W.: J. Urol. **52**:575, 1944.
4. Kelley, J. E., and Hueper, W. C.: Ann. Surg. **88**:1079, 1928.
5. Ferguson, R. S.: Am. J. Roentgenol. **31**:356, 1934.
6. Hamburger, C.; Bang, F., and Nielsen, J.: Acta path. et microbiol. Scandinav. **13**:75, 1936.

follicles. Hypophysectomy does not alter the response of the ovaries.[7] Because of its predominant follicle-stimulating effect, this hormone has been termed follicle-stimulating hormone.

The second type of gonadotropin is biologically identical with the chorionic gonadotropin of pregnancy. In the intact rat or mouse it produces maturation of a few follicles and, in larger doses, formation of corpora hemorrhagica and corpora lutea. In hypophysectomized animals, however, only hypertrophy of the interstitial and thecal cells and luteinization of atretic follicles regularly occur. Maturation of the follicles does not take place, and no corpora lutea are formed unless the hypophysectomy has been recent and at least partially ripened follicles are still present.[7]

In a certain number of cases the titer of chorionic gonadotropin is sufficiently high to give a positive reaction in the Aschheim-Zondek or the Friedman test of the conventional type for pregnancy. The follicle-stimulating factor when employed with the technics and end points of certain investigators[8] and in large quantities will, in the absence of chorionic gonadotropin, give a result interpreted as positive. The demonstration of small quantities of urinary gonadotropins requires the use of extraction and concentration technics. In a number of cases no gonadotropin can be demonstrated.

The urinary excretion of estrogens, progesterone, and androgens has been less extensively investigated.

Gynecomastia is not uncommon in patients with testicular neoplasms. Its pathogenesis is obscure; it may or may not be accompanied by excretion of chorionic gonadotropin and estrogens in high titers.[7b]

CLASSIFICATIONS

Numerous classifications of testicular tumors have been proposed. In general, these have been based on one of two theories of pathogenesis, neither of which is supported by convincing evidence. Ewing[9] stated that (1) all the common testicular tumors are forms of teratoma and (2) the histologically uniform neoplasms represent one-sided developments of teratoma. Chevassu,[10] on the other hand, concluded that the histologically uniform tumor which he named *seminome*, because of its supposed origin from the seminiferous epithelium, is a distinct tumor not arising from teratoma.

---

7. *(a)* Engle, E. T., and Levin, L.: J.A.M.A. **116**:47, 1941. *(b)* Twombly, G. H.: Surgery **16**:181, 1944.

8. Ferguson, R. S.: Am. J. Cancer **18**:269, 1933.

9. Ewing, J.: *(a)* Neoplastic Diseases: A Treatise on Tumors, Philadelphia, W. B. Saunders Company, 1940; *(b)* Surg., Gynec. & Obst. **12**:230, 1911.

10. Chevassu, M.: Tumeurs du testicule, Paris, G. Steinheil, 1906.

In recent years attempts have been made to correlate histologic types of testicular tumors with precise quantitative levels of urinary gonadotropic output.[11] Despite initial enthusiastic reports based on small series, subsequent experience has shown numerous inconsistencies.[12] Correlation with qualitative gonadotropin determinations,[6] on the other hand, has offered greater promise of elucidating the nature of testicular tumors and forming a valid basis for classification. However, until more elaborate studies have been carried out and consistent results obtained by a number of investigators, the retention of pathologic criteria for classifying testicular tumors is necessary.

In the classification which we have adopted on the basis of an analysis of our cases and of those reported in the literature, we have distinguished two main types solely on histologic criteria. Because of its universal use, the term "seminoma" has been retained for the common histologically uniform monocellular neoplasm. The term "teratoma" is used to designate a tumor arising from a cell which has the capacity to form structures normally derived from the three embryonic germ layers. There are two subtypes. One is characterized by the presence of neoplastic structures representing two or more germ layers; the second (the monodermal variety), by a type of tumor tissue foreign to the testis, the presence of which is best explained by regarding it as a one-sided neoplasia of a cell with totipotential capacities.

Because of its widespread acceptance, the artificial subdivision of teratoma into benign and malignant (cancerous) forms has been retained. Addition of the cumbersome prefix "histologically," however, has been unavoidable, since forms which are benign as judged by microscopic criteria are commonly clinically malignant.

Although usually seminoma and teratoma are distinct tumors, they may grow side by side or be intimately mixed with each other. In either event, in order to preserve a simple classification, the impure tumor is regarded as teratoma, since from a clinical point of view the latter tumor carries the graver prognosis.

A third type, the rare interstitial cell tumor, and a heterogeneous group of unusual neoplasms complete the classification.

AUTHORS' CLASSIFICATION

1. Seminoma[10] (embryonal carcinoma of Ewing[9]; embryonal carcinoma with lymphoid stroma of Ewing[9]; spermatocytoma[13]; dysgerminoma[14])

---

11. *(a)* Ferguson.[5] *(b)* Ferguson.[8] *(c)* Hinman, F.; Johnson, C. M., and Carr, J. L.: Tr. Am. A. Genito-Urin. Surgeons **34**:211, 1941. *(d)* Hinman, F., and Powell, T. O.: J.A.M.A., **110**:188. 1938.

12. Twombly.[7b] Colby, F. H.: New England J. Med. **236**:631, 1947.

13. Shultz, O. T., and Eisendrath, D. N.: Arch. Surg. **2**:493, 1921.

14. Meyer, R.: Am. J. Obst. & Gynec. **22**:697, 1931.

2. Teratoma (embryoma[15])
   (a) Histologically cancerous (includes teratocarcinoma and embryonal carcinoma of Friedman and Moore,[16] embryonal adenocarcinoma,[9] and adenocarcinoma[2a]).
   (b) Histologically benign (adult teratoma[9])
3. Interstitial cell tumor
4. Miscellaneous group

SEMINOMA

Pathology.—The typical specimen of seminoma is a circumscribed firm nodular mass which has enlarged and replaced all or the greater part of the involved testicle. Occasionally, seminoma appears as a discrete nodule confined to one region. Its diameter varies considerably (1.5 to 24.0 cm. in the present series); if it arises in a cryptorchid intra-abdominal testicle it tends to attain massive proportions. On section the neoplastic tissue bulges as a homogeneous, moderately soft, grayish white to pink, well demarcated mass, irregularly lobulated by a network of white fibrous septums. Areas of caseation-like necrosis are not infrequent and may be extensive. Large areas of hemorrhage are unusual. Rarely liquefaction takes place, with formation of a cyst.

The epididymis is not infrequently incorporated into the neoplastic mass; less often, invasion of the spermatic cord is encountered. Involvement of these structures may be followed by an outpouring of serous fluid in the tunica vaginalis and eventually this is obliterated by fibrous adhesions.[10] Rarely the parietal tunics and the scrotum are invaded.

Histologically, seminoma is composed of uniform clear polyhedral cells, which are arranged diffusely (fig. 1*A*) or in large or small alveoli isolated by a network of collagenous stroma (fig. 1*B*); acini are not formed. The tumor cells (fig. 2*A*) are characterized by glycogen-rich[9] cytoplasm enclosed by distinct cell borders; depending on the rapidity of fixation, the cytoplasm may have a reticular, granular or empty appearance when stained with eosin-methylene blue or hematoxylin-eosin. The nuclei are distinctive, being uniform, round or oval, single, and centrally placed. One, two, or at times numerous prominent nucleoli lie in the midst of sparse, finely granular chromatin. Mitoses are abundant, varying from 1 to 5 per high power field. Occasionally tumor giant cells with large hyperchromatic nuclei are present. The stroma varies from a delicate network of reticulum in the medullary variety to a framework of broad fibrous septums enclosing nests of tumor cells. It may proliferate to such an extent that the latter are choked out. Dense fibrous scars and a fibrotic response at the periphery of the tumor are not infrequently encountered.

---

15. Wilms, M.: Beitr. z. path. Anat. u. z. allg. Path. **19**:233, 1896.
16. Friedman, N. B., and Moore, R. A.: Mil. Surgeon **99**:573, 1946.

TABLE 1.—*Seminoma*

Case	Age of Patient	Location of Tumor	Presenting Symptoms and Signs	Roentgenologic Data	1. Reaction to Aschheim-Zondek Test 2. Gynecomastia	Location of Metastases and Mode of Spread	Outcome	Size
1	52	R.T.	Epigastric symptoms 4 mo.; weakness and loss of weight; testicular mass 9 yr.	Radiosensitive	1. Test not made 2. Gynecomastia, bilateral, terminal	Abdominal lymph nodes; liver	Dead 6 mo. after hospitalization	Biopsy specimen of omentum*
2	29	L.T.	Tender swelling 5 yr.	0	1. Negative	Epididymis	Living and well 4 11/12 yr. after hospitalization	4 cm.; (located at superior pole of testis)
3	52	R.T.	Testicular swelling 4 mo.; epigastric and thoracic soreness; weakness	Radiosensitive	1. Test not made	Abdominal lymph nodes; liver	Dead 1 yr. after hospitalization	10 x 6 cm.
4	39	L.T.	Testicular swelling 3 yr.; rapid increase 4 mo.	0	1. Negative	0	Living and well 10½ yr. after hospitalization	5 x 4 x 3.5 cm.
5†	27	L.T.	Testicular swelling 3 wk.	Radiosensitive	1. Negative	Widespread hematogenous and lymphatic metastasis‡	Dead 1 1/12 yr. after orchidectomy	80 Gm.
6†	30	L.T.	Testicular swelling 2 9/12 yr.; pain	Radioresistant (secondary infection present)	1. Test not made	Postoperative recurrence: scrotum and inguinal lymph nodes	Dead 10 mo. after orchidectomy	8 x 5 x 2 cm.
7	47	R.T.	Testicular swelling 1 yr.	Prophylactic	1. Test not made	Epididymis	Living and well 20 11/12 yr. after hospitalization	6 cm.
8	44	R.T. (pelvic)	Pain in groin 2 yr.; dysuria 1 week	1. Prophylactic 2. Radiocurative	1. Negative	Lung, liver	Living and well 18 8/12 yr. after hospitalization	700 Gm., 12 x 8 cm.

9	37	L.T.	Testicular swelling 1¼ yr.	Prophylactic	1. Test not made	Left cervical lymph nodes	Dead 5 mo. after hospitalization	300 Gm., 11 x 7.5 x 6 cm.
10	49	L.T.	Testicular swelling 10 mo.	0	1. Test not made	0	Living and well 2 5/12 yr. after hospitalization	118 Gm., 7.5 x 4.5 cm.
11	75	R.T. (pelvic)	Nocturia 1 yr.; loss of weight; asthenia; ankle edema 6 mo.	0	1. Test not made	Retroperitoneal lymph nodes; peritoneum‡	Dead 1 mo. after hospitalization	1,800 Gm, 24 x 13 x 10 cm.
12	40	L.T.	Testicular swelling and hardness 3 mo.	Prophylactic	1. Negative	0	Living and well 12 11/12 yr. after hospitalization	1.9 x 1.4 cm.; (located at inferior pole of testis)
13	37	L.T.	Testicular swelling 1½ yr.; lumbar pain and fatigue 2 mo.	Radiosensitive	1. Negative	Abdominal and left supraclavicular lymph nodes; liver	Dead 8 mo. after hospitalization	700 Gm., 13 cm.
14	32	R.T.	Testicular swelling 7 yr.; recent increase 2 yr.	Prophylactic	1. Doubtful	0	Living and well 8 7/12 yr. after orchidectomy	10 x 7.5 cm.
15	28	L.T.	Testicular mass 2½ yr.; recent increase for several weeks.	0	1. Negative	0	Living and well 1 1/12 yr. after orchidectomy	1 cm.; (located at superior pole of testis)
16	40	L.T.	Testicular swelling 3 yr.	0	1. Test not made	0‡	Dead 4 1/12 yr. after orchidectomy; no evidence of tumor at autopsy	11 x 8 x 7 cm.
17	48	Scrotal testes normal to palpation	Edema and pain in legs; loss of weight; costovertebral pain on right 4 mo.	Radiosensitive	1. Test not made	Widespread hematogenous and lymphatic metastasis	Dead 1 5/12 yr. after hospitalization	Biopsy specimen: lymph node*

*The primary tumor was not examined.
‡The patient was admitted with metastases after treatment had been given in another hospital
‡Autopsy was performed.

The majority of specimens show a variable degree of lymphocytic infiltration of the stroma. This may extend to the finest ramifications of the stromal network. Lymphoid nodules may form. Occasionally plasma cells and eosinophilic leukocytes are present among the infiltrating

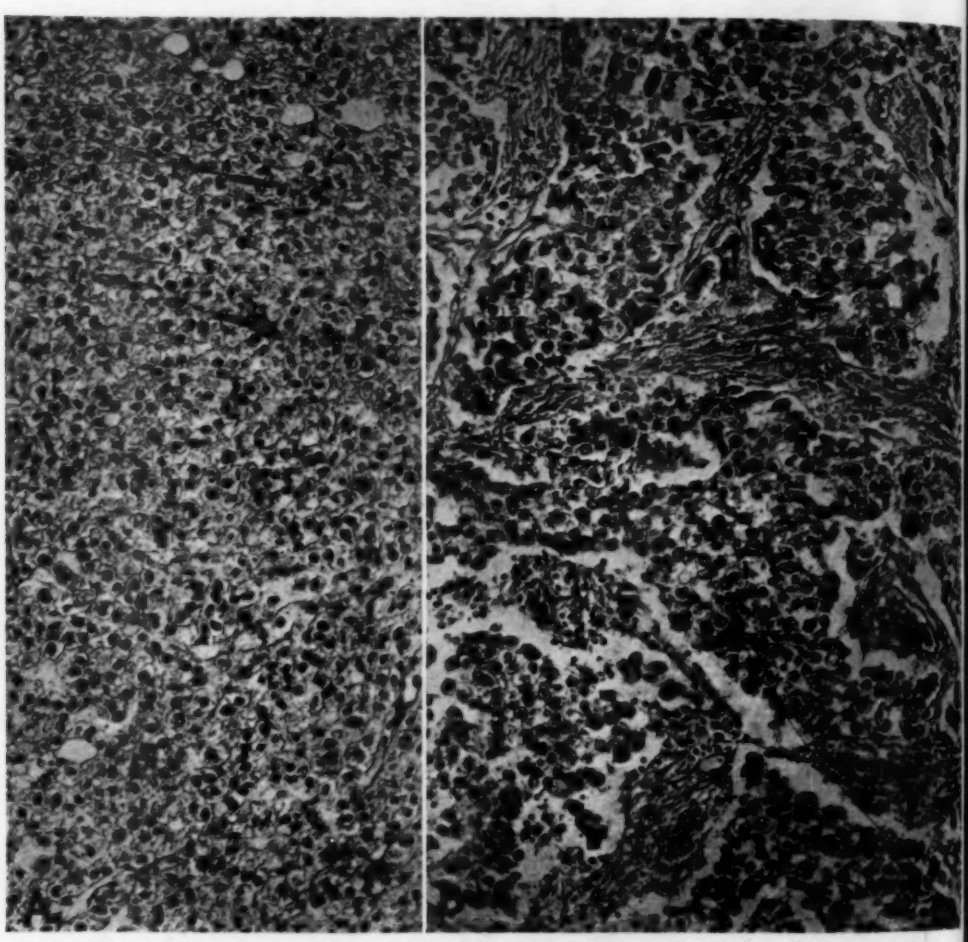

Fig. 1.—*A*, omental metastasis of seminoma (case 5) showing a diffuse pattern; x 200. *B*, seminoma (case 8) showing an alveolar pattern; x 200.

cells. In certain specimens the lymphocyte element of the stroma is quite prominent (fig. 2*B*). This has led Ewing[9] to subdivide seminoma into embryonal carcinoma and embryonal carcinoma with lymphoid stroma. Neither of these subdivisions, however, has proved to represent a clinical or a pathologic entity.

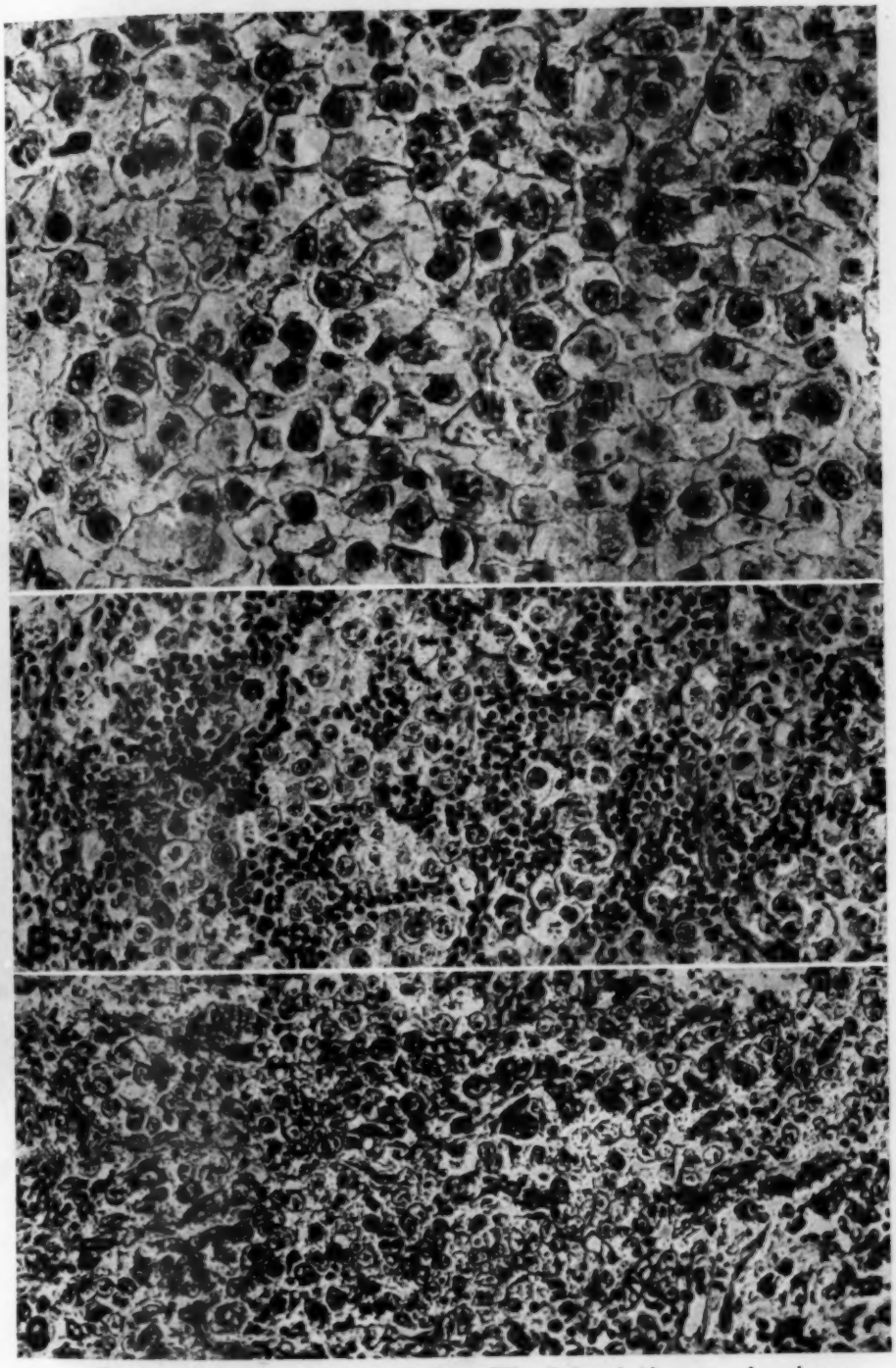

Fig. 2.—*A*, cells of seminoma (case 7); x 779. *B*, lymphoid stroma of seminoma (case 4); x 346. *C*, omental metastasis of seminoma (case 1) showing large collections of epithelioid cells characterized by abundant, deeply staining cytoplasm among the smaller pale seminoma cells; x 346.

A striking feature of the stroma of seminoma, rarely observed in other neoplasms, is the frequent prominence therein of epithelioid cells (fig. 2*C*). These may occur either in unorganized collections or in lesions simulating tubercles. Giant cells of the Langhans variety may be formed.

Massive caseation-like necrosis of the tumor is commonly observed microscopically. Occasionally, the necrotic zones are bordered by rows of phagocytes laden with lipoid substance.

Reliable criteria for judging by microscopic examination whether a variety of seminoma is cancer have not been established, for despite the constant histologic appearance of cancer, there will be an unpredictable number of cases in which it will behave clinically as benign.

In view of the fact that seminoma occurs exclusively in gonadal tissue, it is logical to trace the origin of the neoplasm to sexual cells or to their embryonic predecessors. Chevassu's theory of origin from spermatogonia is based on the superficial resemblance of the neoplastic cells to the latter and the occasional observation of seminoma growing and apparently originating within testicular tubules. Study of seminoma as it occurs in animals lends support to Chevassu's theory.[17] On the other hand, the fact that it occurs additionally in the ovary (dysgerminoma), where it is not infrequently associated with pseudohermaphroditism, favors an origin from the primordial (neuter or undifferentiated) germ cell.[14]

Endocrinology.—The results of the conventional Aschheim-Zondek and Friedman tests are most commonly negative in cases of seminoma (in the present series they were negative in 7 cases, doubtful in 1 case and positive in none). This is in accord with the tendency of the tumor to be associated with absence of excretion of gonadotropin or with excretion of amounts so small as to be demonstrable only by quantitative technics.[7b] The gonadotropin characteristically excreted is the follicle-stimulating one.[6] Its significance is not clear, for (1) it cannot be recovered biologically from the tumor tissue (hence is apparently of pituitary origin)[6] and (2) its level may remain elevated for months or years despite the removal of the primary tumor and absence of metastases.[18] Although its presence has been considered pathognomonic of seminoma,[6] it has been reported to be present in increased titers in occasional cases of teratoma; likewise, certain tumors diagnosed pathologically as seminomas have been associated with excretion of chorionic gonadotropin.[7b] Hence before one can justifiably draw conclusions concerning the endocrinologic aspects of seminoma, analyses of large series correlating qualitative and quantitative gonadotropin determinations with the results of exhaustive histologic study are necessary.

17. Peyron, A.: Bull. Assoc. franc. p. l'etude du cancer **25**:103, 1936.
18. Dean, A. L.: J.A.M.A. **105**:1965, 1935.

The tendencies of seminoma to be associated with normal urinary levels of estrogen and decreased levels of androgen have been reported.[19]

Clinical Aspects.—In the present series seminoma constituted 46 per cent of testicular tumors. In other series[20] the percentage incidence has ranged from 46 to 70. The age incidence is generally stated to be maximum in the fourth decade.[2e] Above 50 the occurrence of seminoma is infrequent; below 20, extremely rare. Friedman and Moore,[16] most of whose patients were 18 to 38 years of age, and whose over-all percentage incidence of seminoma was 35, observed a strikingly higher incidence in cryptorchid testes (80 per cent).

Testicular enlargement was the presenting symptom in 12 of 14 cases of seminoma of the scrotal testis in the present series. Pain or tenderness was noted but twice. The duration of testicular symptoms in the entire group of 14 cases varied from three weeks to nine years; in 10 it was one year or more, and in 6, three years or more. In 2 cases the tumor occurred in a right intra-abdominal testis (cases 8 and 11). In case 17 a large abdominal mass was palpable. Both testes were in the scrotum and were not abnormal to palpation. Biopsy of a lymph node showed indisputable seminoma. Death, due to metastases, occurred seventeen months after the initial hospitalization. Permission to perform an autopsy was not obtained. This case may be one of seminoma occurring in a rest of testicular tissue misplaced during embryonic migration; a more probable explanation, however, is the presence of a scrotal testicular neoplasm which failed to grow or retrogressed despite progression of its metastases.

Excluding 2 cases in which clinical or pathologic studies were inadequate for interpretation, 15 cases fell into three clinical groups.

Group I comprised 8 cases of intrascrotal tumor from which no metastases occurred and in which orchidectomy with or without prophylactic roentgen therapy resulted in cure. In these cases the tumor tended to be of small size despite a frequent history of long duration. In 2 cases the size of the tumor had been stationary over a number of years but had recently increased.

Group 2 comprised 5 cases of clinically "malignant" intrascrotal seminoma in which metastases were present at the time of, or within three months after, the initial admission. All were fatal. In general, in these cases the tumor was rapidly growing, tending to attain a large size in a short time. In 1 instance (case 1), however, a mass in the region of the testis was said to have been present for nine years.

---

19. Hamburger, C., and Godtfresden, E.: Acta path. et microbiol. Scandinav. 18:485, 1941.

20. Chevassu.[1e] Nash and Leddy.[5e]

Group 3 comprised 2 cases of tumor of an intra-abdominal testicle. In both instances the tumor was of large size. One case (no. 11) was fatal, owing to the production of hydronephrosis by metastases of the tumor; in the second instance (case 8) the bearer of the tumor is alive eighteen years and eight months after removal of the tumor and roentgen therapy.

The incidence of metastases in cases of seminoma is controversial. Chevassu[10] stated that it was rare for the tumor to extend beyond the lumbar lymph nodes and become generalized. Friedman and Moore,[16] who studied 319 cases, few of which were followed for more than one year, found metastases in less than 10 per cent. These were usually restricted to the retroperitoneal tissues or the peritoneal linings. Nash and Leddy,[2c] in contrast, noted metastases at the time of first examination at the Mayo Clinic in 46 per cent of cases of seminoma. The abdominoaortic and inguinal lymph nodes were the most frequent sites; the lungs, however, were involved in only 2 per cent of cases. In the present series, if one excludes patients who received primary treatment elsewhere and entered this hospital subsequently because of the presence of metastases, 4 of 14 patients had metastases at or within one month after entry; in 2 of the remaining 10 the tumor metastasized subsequently. Usually involvement of the abdominal or cervical lymph nodes gave the first indication of tumor spread; terminally, widespread blood-borne metastases were common. The histologic structure of the metastases, in all cases examined, was identical to that of the primary tumor.

The striking roentgen ray sensitivity of seminoma has been noted by numerous authors.[21] In the present series there were 6 cases in which metastases were irradiated, and in all 6 these showed an initial marked decrease in size. In 1 case (no. 8) metastatic growths in the right lung and in the liver[22] disappeared permanently (sixteen year period of observation). In the remaining 5 cases, the metastases eventually grew rapidly, "escaping" from the effects of irradiation. The experience of other authors[23] has been more encouraging with regard to the value of roentgen therapy.

If one excludes patients who received primary treatment elsewhere, 9 of 15 patients subjected to orchidectomy with or without

---

21. *(a)* Hinman, F.: J. Urol. **34**:72, 1935. *(b)* Hamburger, Bang and Nielsen.[9] *(c)* Nash and Leddy.[2c] *(d)* Pendergrass, E. P.; Chamberlin, G. W.; Selman, J., and Horn, R. C., Jr.: Am. J. Roentgenol. **55**:555, 1946. *(e)* Gordon-Taylor, G., and Till, A. S.: Brit. J. Urol. **10**:1, 1938.

22. The liver was greatly enlarged and tender. The right lung showed by roentgenogram two large areas of metastatic growth. Less than three months after the institution of roentgen therapy the liver was no longer palpable and the lungs showed no roentgenologic evidence of metastatic disease.

23. Cabot, H., and Berkson, J.: New England J. Med. **220**:192, 1939.

irradiation were alive without metastases or had died of unrelated causes nine months to twenty-one years later. Of the 8 who died (in the entire series), 6 died in one year or less after seeking medical treatment. These findings attest to the relatively good prognosis of seminoma and are in accord with the experience of numerous other investigators.[24]

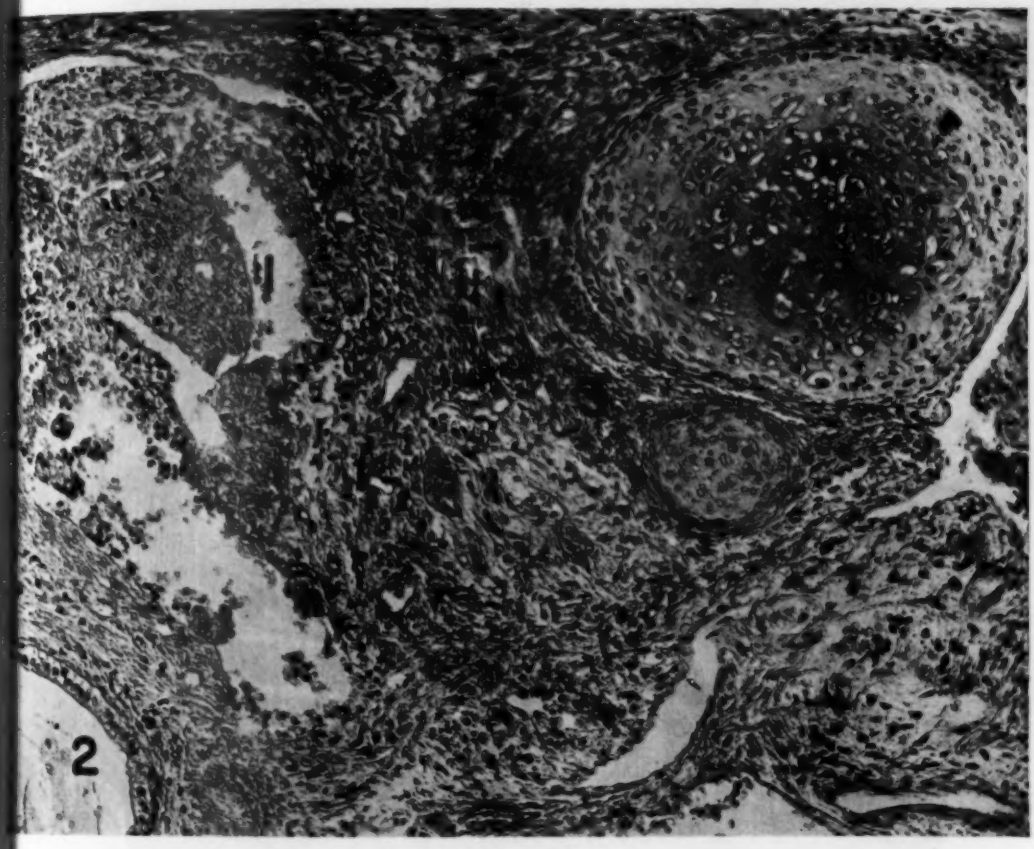

Fig. 3.—Teratoma (case 24) showing carcinoma *(1)*, a cyst lined by vacuolated simple columnar epithelium *(2)* and islands of cartilage *(3)*; x 200.

HISTOLOGICALLY CANCEROUS TERATOMA

Pathology.—The typical specimen of histologically cancerous teratoma is a circumscribed firm mass which has enlarged and replaced all or the greater part of the testicle. Its size varies greatly.

On section the neoplastic tissue bulges as a soft, at times friable, multicolored mass. The gray of the viable tumor is commonly mottled with, or obliterated by, extensive red to brown areas of hemorrhage.

24. Hamburger, Bang and Nielsen.[8] Chevassu.[10] Nash and Leddy.[2c] Friedman and Moore.[16] Gordon-Taylor and Till.[21e]

Yellow foci of necrosis and white bands of collagenous tissue are common. Often, cartilage or multiple cysts of teratomatous origin are recognizable.

The epididymis and the spermatic cord are not infrequently invaded. Adhesions between the parietal and the visceral tunica are observed with greater frequency than in the seminoma.[9] Rarely a hemorrhagic hydrocele occurs.

Microscopically, the tumor is composed of an intimate mixture of teratomatous and cancerous neoplastic tissues (fig. 3).

In the vast majority of cases (100 per cent in the present series; 92.5 per cent in the series of Friedman and Moore[16]) the cancerous element in at least portions of the tumor takes the form of a distinctive embryonal epithelial neoplasia, variously named (1) embryonal adenocarcinoma,[9] (2) adenocarcinoma[25] and (3) embryonal carcinoma.[16] Terms 1 and 2 are inadequate since the adenomatous form is but one variant of the tumor; term 3, the most accurate, must be avoided since it is used by numerous authors synonymously with "seminoma." For convenience, the least controversial term, "embryonal adenocarcinoma," will be employed in the present discussion. A subvariety of the embryonal adenocarcinoma is the chorionic carcinoma.

Microscopically, the embryonal adenocarcinoma is characterized by a striking variation in pattern from tumor to tumor and indeed from area to area of the same tumor. It is commonly composed of elongated winding acini with stratified linings. A pattern of papillary adenocarcinoma is frequent (fig. 4*A*). On the other hand, the tumor cells may grow in broad sheets, nests or anastomosing trabeculae; these may be perforated by numerous oval to elongated slitlike lumens. Finally, a common pattern is the reticular in which the cells are so arranged along a connective tissue network as to enclose large round or oval spaces (fig. 6*A*).

The tumor cells vary from area to area as greatly as the pattern. They are commonly large, irregular, columnar, with clear to faintly granular cytoplasm and sizable nuclei containing prominent nucleoli (fig. 4*B*). On the other hand, they may be uniformly small and anaplastic or may contain huge irregular hyperchromatic nuclei. Occasionally, they closely resemble or are indistinguishable from cells of the seminoma, but their tendency to orient themselves about oval or elongated spaces enables one to identify them.

Commonly encountered are masses of uniform oval or polyhedral cells characterized by clear or pale granular cytoplasm; their nuclei may be large and oval with one or more prominent nucleoli (fig. 5*A*) or smaller and round, enclosing coarse clumps of chromatin (fig. 5*B*).

25. *(a)* Gordon, W. G.: J. Urol. **43**:851, 1940. *(b)* Nash and Leddy[3e] *(c)* Hinman.[3a]

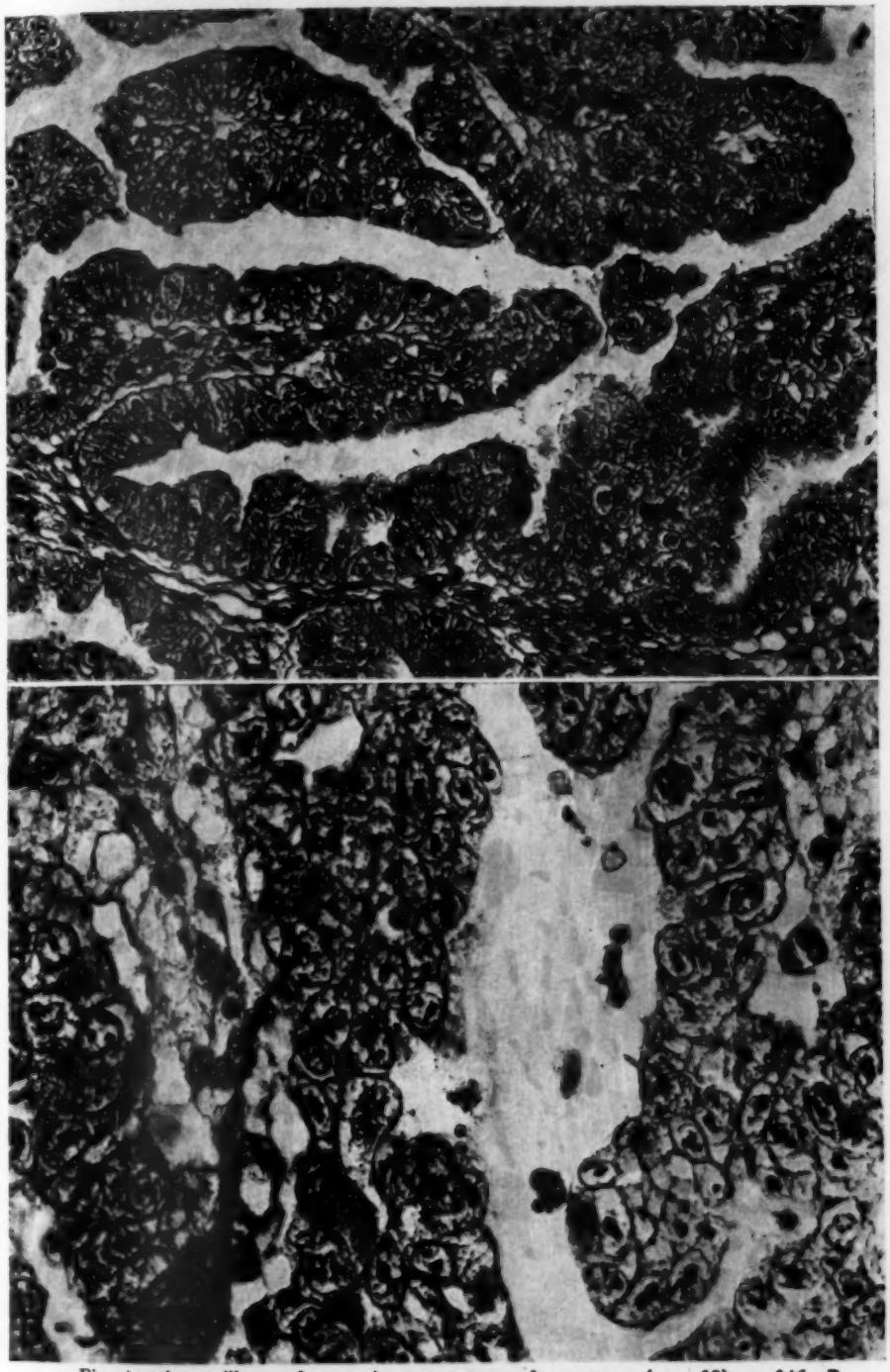

Fig. 4.—*A*, papillary adenocarcinoma pattern of teratoma (case 30); x 346. *B*, higher magnification (x 779) of papillary adenocarcinoma in the same case. Contrast the cytologic features with those shown in figure 2*A*.

TABLE 2.—*Teratoma*

Case	Age of Patient	Location of Tumor	Presenting Symptoms and Signs	Roentgenologic Data	1. Reaction to Aschheim-Zondek Test 2. Gynecomastia	Location of Metastases and Mode of Spread	Outcome	Size
18	31	L.T.	Testicular pain and swelling 5 mo.	0	1. Test not made	Abdominal lymph nodes	"Huge" metastases 5 mo. after hospitalization	7 x 5 cm.
19	31	R.T.	Testicular swelling 2 mo.	0	1. Positive	0	Living and well 2 3/12 yr. after hospitalization	9 x 7 x 5 cm.
20	48	R.T.	Testicular pain and swelling, recurrent, 10 wk.	?	1. Test not made	Spermatic cord; lungs; liver	Dead 5 mo. after hospitalization	102 Gm.*
21	24	L.T.	Testicular pain and tenderness 1½ mo.	Radiosensitive	1. Negative	Abdominal lymph nodes; inferior vena cava; lungs†	Dead 10 mo. after hospitalization	3 x 2.5 cm.
22	47	R.T.	Testicular pain 8 mo.; hardness 5 mo.	0	1. Test not made	0	Living and well 20 1/12 yr. after hospitalization	8 cm.
23	38	R.T.	Recurrent testicular swelling and pain 6 mo.; recent increase 2 wk.	0	1. Test not made	Lungs	Dead 10 mo. after hospitalization	7 x 5 x 3.5 cm.*
24	24	L.T.	Testicular swelling 2 mo.; pain, tenderness	Radioresistant	1. Test not made	Lung and elsewhere	Dead 3 yr. after hospitalization	6.3 x 4 x 2.8 cm.*
25	29	R.T.	Testicular tenderness; mass 3 wk.	Radioresistant	1. Test not made	Lungs: mediastinal and abdominal lymph nodes†	Dead 9 3/12 yr. after hospitalization	4 x 4 x 3 cm.

26	29	R.T.	Testicular swelling 7 wk.	Radioresistant	1. Negative 2. Gynecomastia, bilateral	Widespread hematogenous and lymphatic spread†	Dead 11 mo. after hospitalization	4.5 cm.; (at inferior pole of testis)
27	34	L.T.	Testicular swelling 5 mo.; pain 3 wk.	Radioresistant	1. Positive	Lungs	Dead 2 mo. after hospitalization	7 x 4 x 4 cm.*
28§	30	R.T.	Testicular swelling 6 mo.	0	1. Test not made	Recurrence in groin; liver	Dead 1 10/12 yr. after orchidectomy	10 cm.*‡
29§	17	R.T.	Testicular swelling 4½ yr.	Radioresistant	1. Positive	Widespread hematogenous and lymphatic spread†	Dead 9 mo. after orchidectomy	10 x 8 cm.
30	20	R.T.	Testicular swelling and soreness 2 mo.	Radioresistant	1. Negative	Abdominal lymph nodes	Dead 2 yr. after hospitalization	5.3 x 3.5 x 2.7 cm.
31	24	R.T.	Testicular pain and swelling 2 mo.	0	1. Positive; postoperatively negative	Epididymis?	Living and well 10 mo. after hospitalization	6.5 cm.
32	22	L.T. (inguinal)	Testicular swelling 14 mo.; anorexia; cough; cervical and abdominal masses 2 wk.	Radioresistant (initial slight radiosensitivity)	1. Positive 2. Bilateral gynecomastia	Widespread hematogenous and lymphatic spread†	Dead 2 mo. after hospitalization	10 x 6.5 x 5 cm.
33§	31	R.T.	Testicular swelling, lumpiness 1 yr.	0	1. Test not made	Widespread hematogenous and lymphatic spread†	Dead 1 yr. after orchidectomy	Metastases‡

*Seminoma was present in the tumor.
†Autopsy was performed.
‡The primary tumor was not examined.
§The patient was admitted with metastases after treatment had been given in another hospital

Such masses of cells are identical in appearance with the cytotrophoblast of the placenta. Smudgy small cells with densely eosinophilic cytoplasm and dark nuclei may lie compressed between these groups of cells. This constitutes the applique pattern described by Friedman and Moore,[16] the applied cells representing poorly formed syncytiotrophoblast (fig. 5*A*). Well formed syncytiotrophoblastic elements are commonly encountered (in 11 of 16 cases in the present series). These consist of giant multinucleated masses of dense homogeneous or finely granular cytoplasm enclosing vacuoles and blood-filled lacunas. Frank chorionic carcinoma, in which masses of cytotrophoblast are found in intimate association with syncytiotrophoblast, was present in a number of cases (fig. 5*B*).

Embryonic disks composed of inner cell mass, amnion and trophoblast are occasionally encountered.[26]

The connective tissue intimately associated with the embryonal adenocarcinoma is distinctive, frequently exhibiting teratoid potentialities. It is characterically quite cellular, the cells often becoming organized focally to form dense cellular clusters, or nests of myxomatous tissue, embryonic cartilage, or smooth muscle.

Extensive hemorrhage and necrosis are common features. They may be accompanied by widespread overgrowth of granulation and scar tissue. Frequently, and characteristically in areas of chorionic carcinoma, dilated, thin-walled vascular channels are present (fig. 5*B*).

Unusual cancerous elements encountered in the present series were fibrosarcoma, neuroepithelioma and mesonephroma. The first was present focally in 2 cases, enclosing acini lined by anaplastic epithelial cells. In a third case a large portion of the tumor was composed of fibrosarcoma enclosing epithelial acini as well as numerous rosettes containing prominent blepharoplasts (fig. 7). In 2 final cases large peg-shaped cells were growing to form a network of spaces into which glomerulus-like structures protruded, creating a pattern indistinguishable from the ovarian mesonephroma[27] (fig. 6*B*).

The literature contains reports of numerous rare cancerous elements observed in testicular teratoma. These include squamous cell carcinoma,[28] basal cell carcinoma[28] and rhabdomyosarcoma.[25a] The cases of chondrosarcoma of Pick and Kocher[9a] are best interpreted as cases of cancerous teratoma in which the nonsarcomatous elements have been overgrown.

The teratomatous elements that we shall describe as occurring in the histologically benign teratoma are commonly present in varying amounts.

---

26. Peyron, A.; Limousin, H., and LaFay, B.: Bull. Assoc. franc. p. l'etude du cancer **25**:851, 1936.

27. Schiller, W.: Am. J. Cancer **35**:1, 1939.

28. Bell, F. G.: Brit. J. Surg. **13**:7, 1925.

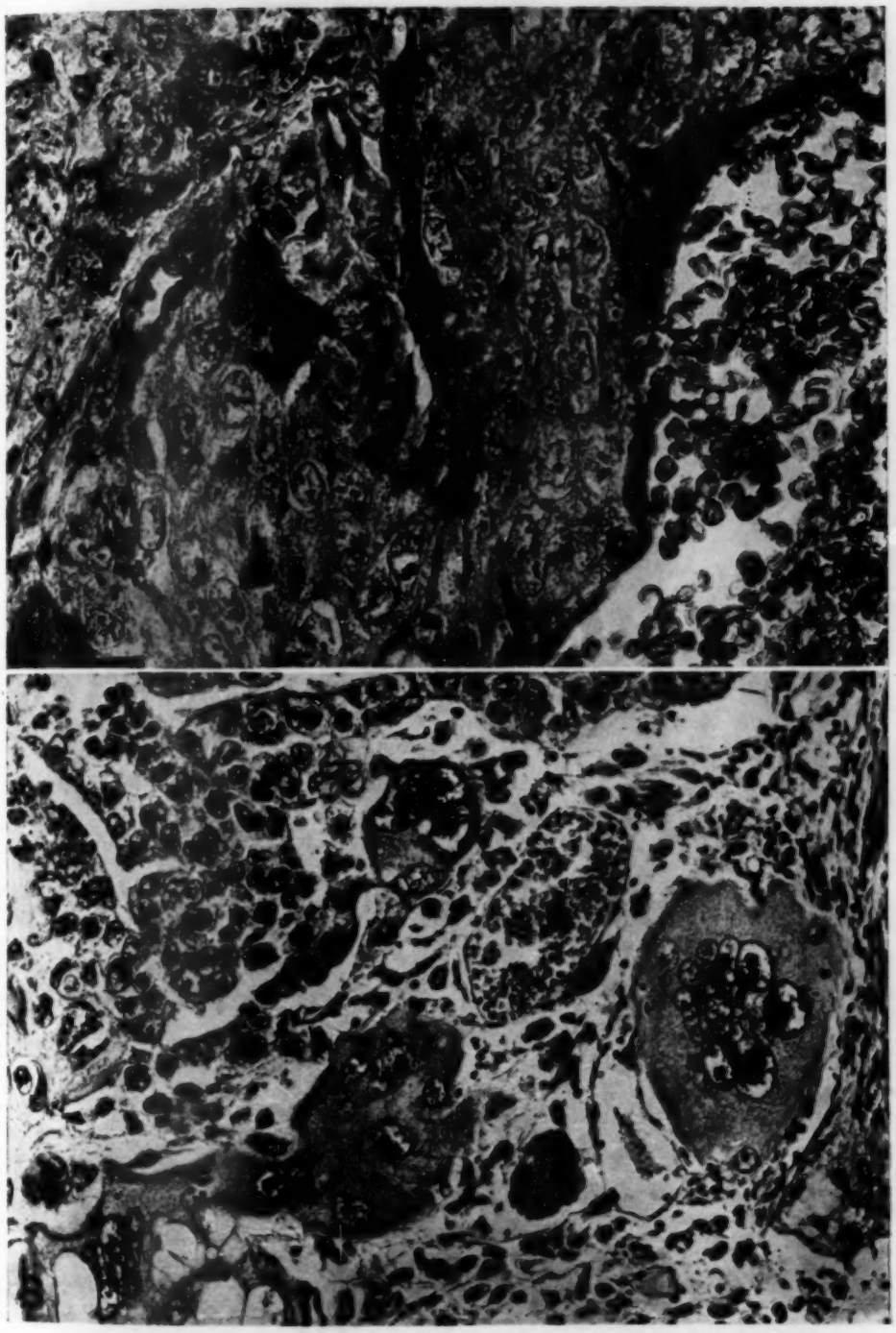

Fig. 5.—*A*, applique pattern of teratoma (case 27); x 713.5. The cytologic aspects of the applied cells are best visualized just above center. The cytotrophoblast shows characteristic oval nuclei and pale granular cytoplasm. *B*, pulmonary metastasis of teratoma (case 33) illustrating occurrence of chorionic carcinoma; x 357. Typical cytotrophoblast appears in the left upper quadrant. Note the dilated vascular channels associated with the syncytiotrophoblast and the vacuolation of the latter.

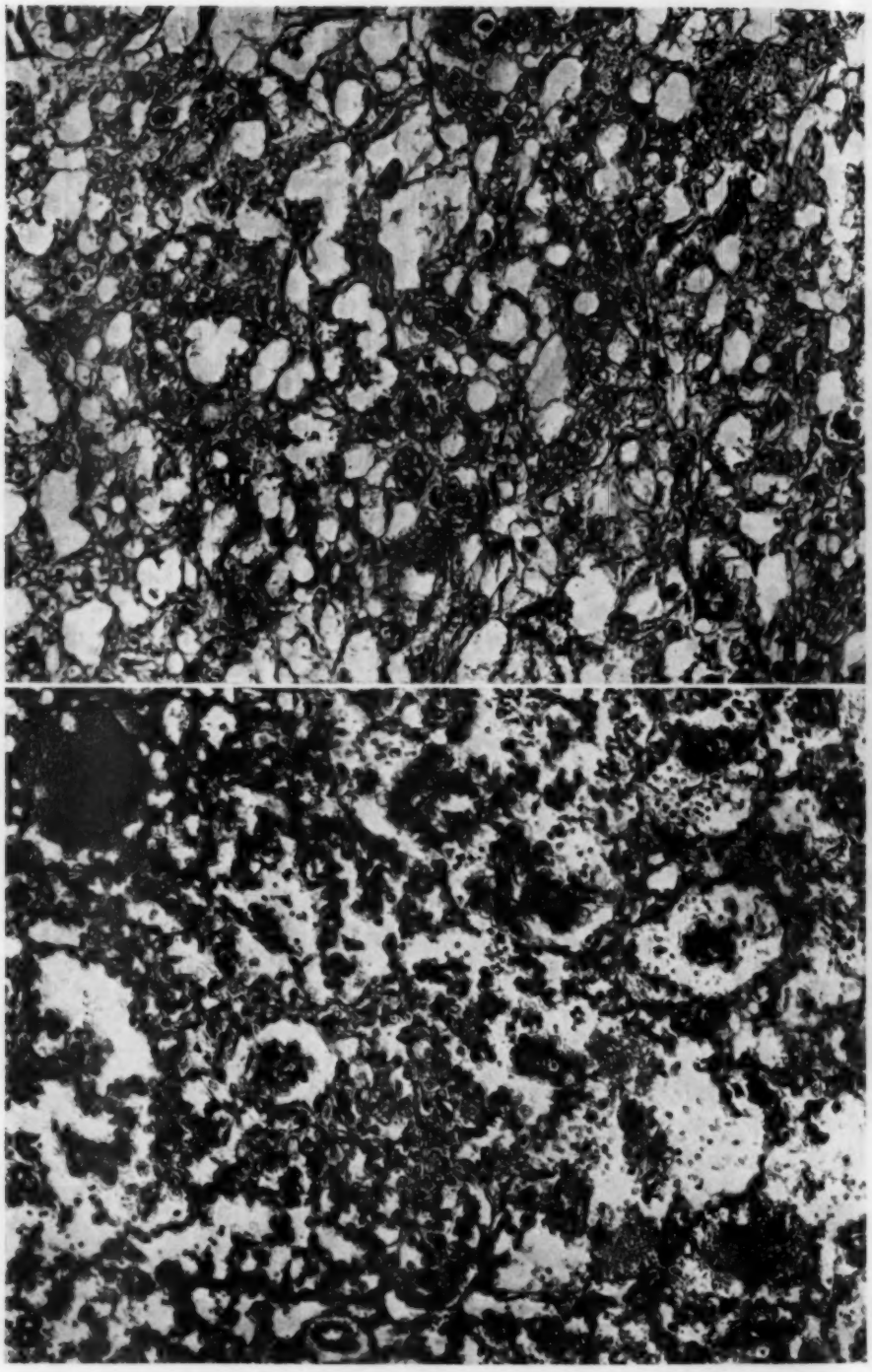

Fig. 6.—*A*, reticular pattern of teratoma (case 30); x 336. *B*, inguinial metastasis of teratoma (case 28) showing a mesonephroma pattern; x 168.

Seminoma: Areas of seminoma are not infrequently observed in cases of histologically cancerous teratoma. In the present series seminoma was present in 5 of 16 cases; in 3 it was well circumscribed. In 1 case cancerous teratoma and seminoma were forming grossly distinct nodules side by side. In 2 cases, on the other hand, there was an intimate mixture of seminoma, mesoblastic teratomatous elements and embryonal adenocarcinoma, but even here the seminomatous foci retained their

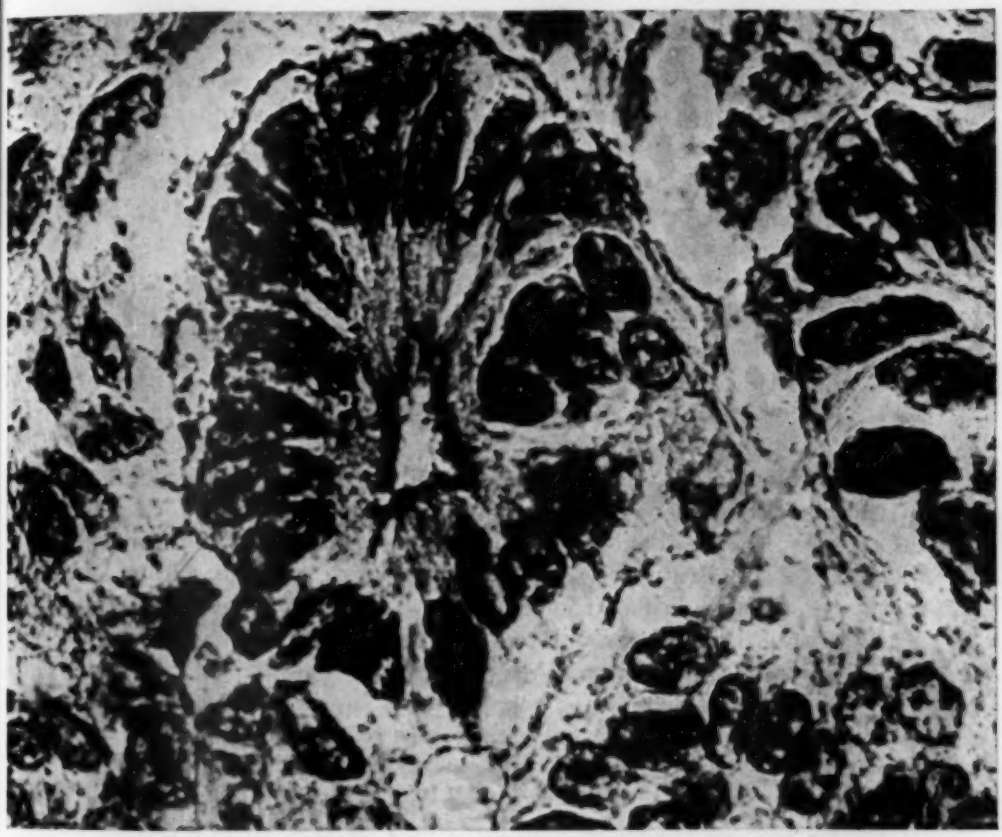

Fig. 7.—Teratoma (case 23) showing a neuroepithelial rosette; x 1,800. Blepharoplasts are not distinct.

distinctive features, the cells nowhere appearing to "differentiate" into embryonal adenocarcinoma.

Embryonal Adenocarcinoma: Numerous authors[29] have segregated from cases of histologically cancerous teratoma those cases in which the microscopic sections examined indicate that the tumor is pure embryonal adenocarcinoma, teratomatous elements being absent. We

29. Friedman and Moore.[15] Gordon.[25a]

have chosen to regard these cases of pure embryonal adenocarcinoma as cases of teratoma for: 1. The absence of teratomatous elements may indicate merely an inadequate number of sections. In the present series, teratomatous elements were present in every tumor showing embryonal adenocarcinoma, although in 2 cases numerous sections failed to show more than one or two tiny areas of frank teratomatous differentiation. 2. The teratoid potentialities of the connective tissue component of the embryonal adenocarcinoma and the common intimate intermingling of the latter with well formed teratomatous elements bespeak a fundamental relationship between the two. 3. This relationship is supported by the finding that cancerous teratoma frequently metastasizes as embryonal adenocarcinoma; in addition, in a small number of cases the reverse is true.[16]

The most satisfactory interpretation of the aforementioned observations is that the cells of embryonal adenocarcinoma are immature epithelial descendants of a totipotential cell which commonly reveals its teratomatous capacities elsewhere in the neoplasm. The higher degree of clinical malignancy of pure embryonal adenocarcinoma is in keeping with its lack of differentiation.[16]

Chorionic Carcinoma: Because of the lack of clearcut histologic criteria for the identification of cytotrophoblast and because large numbers of sections may be required to demonstrate a small focus of trophoblastic cells, the exclusion of chorionic carcinoma in a case of embryonal adenocarcinoma is not justified by histologic study alone. Logically, the production of chorionic gonadotropin, estrogens or progesterone by the tumor (see subsequent section on endocrinology) is the best positive criterion for diagnosis. The absence of demonstrable amounts of these factors indicates, then, either an insufficient amount of viable tumor to form measurable quantities or, possibly, a lack of functional differentiation of the tumor tissue. In the present series the correlation between the histologic presence of chorionic carcinoma and the results of the Aschheim-Zondek test was poor. This is understandable in view of the fact that large numbers of microscopic sections of the tumors were not examined.

Testicular teratoma may arise from (1) totipotent sex cells or their embryonic predecessors undergoing a type of neoplasia akin to parthenogenesis[9] or (2) intratesticular inclusions of totipotent primitive embryonic cells (blastomeres). The former origin is far more likely in view of the predilection of teratoma for the sex glands and the distinctive features of teratoma in this location as compared with extragenital teratoma.[9]

The occasional coexistence of teratoma and seminoma is not surprising in view of the origin of both types of tumor from cells of the

sex series. The precise histogenetic relationship between the two, however, is at present obscure. For their association when they form distinct tumors growing adjacent to each other Peyron[30] offered an alternative explanation. He reported 5 cases of seminoma arising in a zone of testicle invaded or compressed by a tumor diagnosed as malignant embryoma; in 2 of the cases the seminoma was confined to the testicular tubules. These observations suggested that the growth of seminoma may be stimulated by the presence of an active principle elaborated by teratoma.

Endocrinology.—Histologically cancerous teratoma is characteristically associated with excretion of large amounts of gonadotropins, the tumors containing appreciable amounts of trophoblastic neoplastic tissue giving the highest titers.[31] Most commonly the gonadotropin is of the chorionic type,[6] but occasionally the follicle-stimulating type is found alone or in combination with the former. In a small number of cases no excretion of either type is demonstrable. Chorionic gonadotropin has been demonstrated biologically in, and hence is apparently produced by, the tumor tissue.[6] The results of the conventional Aschheim-Zondek and Friedman tests for pregnancy are frequently positive (in 5 of 8 cases in the present series).

The estrogen excreted in the urine may or may not be increased.[32] Excretion of progesterone has been reported in 1 case of chorionic carcinoma of the testis.[7b] The latter findings are not surprising since normal syncytiotrophoblast apparently secretes both estrogens and progesterone.[33]

Clinical Aspects.—Histologically cancerous teratoma constituted 43 per cent of the testicular tumors in the present series. The percentage incidence reported elsewhere[20] has varied from 34.5 to 51. The maximum age incidence is found in the third decade; in the series of Scheetz and Leddy,[34] 54 per cent occurred during this period and 93 per cent between the ages of 15 and 39.

Testicular enlargement was the presenting symptom in 15 of the 16 cases in the present series. One tumor occurred in an undescended inguinal testicle. Pain or tenderness on physical examination was, in contrast to seminoma, common, occurring in 9 cases. This may be attributed to the more rapid growth and to the greater frequency of extensive hemorrhage in this type of tumor. The duration of symptoms varied from three weeks to four and one-half years; in 12 cases it was

30. Peyron, A.: Bull. Assoc. franc. p. l'etude du cancer **25**:422, 1936.

31. Ferguson (footnotes 5 and 8). Hinman and Powell.[11d]

32. Hamburger and Godtfresden.[19] Smith, G. V., and Smith, O. W.: Proc. Soc. Exper. Biol. & Med. **32**:847, 1935. Twombly.[7b]

33. Wislocki, G. B., and Bennett, H. S.: Am. J. Anat. **73**:335, 1943.

six months or less, and in 6, two months or less. No correlation could be discovered between the size of the tumor, the duration of symptoms and the prognosis; nor did the cases lend themselves to clinical grouping.

Numerous authors have observed that histologically cancerous teratoma tends to metastasize rapidly and that early blood stream dissemination is frequent. Sheetz and Leddy,[34] with a series of 54 cases, reported that the presenting symptom was due to metastases in 7 and that 17 of 37 patients not previously treated had demonstrable metastases at the time of hospitalization. In the present series (excluding cases in which primary treatment was received elsewhere) 6 of 13 patients had metastases at or within one month after entering the hospital. Of 6 remaining who have been followed for one year or more, metastases subsequently developed in 4. The striking incidence of blood-borne metastases is shown by the finding that of the 13 patients (including all cases) in whom metastases developed, 9 had pulmonary involvement at the time metastases were first detected clinically.

In all cases, including 6 in which unrestricted autopsy was performed, the histologic composition of the metastases examined was similar to that of the primary tumor, although variations in the proportions of teratomatous, chorionic and carcinomatous elements were observed.

The radioresistance of cancerous teratoma has been noted by numerous authors.[35] Sheetz and Leddy,[34] in an analysis of 54 cases, concluded that roentgen therapy had, in spite of occasional transitory improvements, no beneficial effect. In the present series, of 7 patients in whom the results of irradiation were capable of evaluation, 6 showed resistant or only slightly sensitive lesions. One (case 21) showed initial marked regression of pulmonary metastases, but subsequently there were radioresistant recurrences.

According to Chevassu,[10] histologically cancerous teratoma has a uniformly poor prognosis. Of 13 cases reported by Hamburger, Bang and Nielson,[6] 12 were fatal within seven months. Gordon-Taylor and Till [21e] reported 12 fatalities in 14 cases. Of 36 patients reported by Sheetz and Leddy,[34] all receiving prophylactic roentgen therapy, 8 survived five years. Of 13 patients in the present series (excluding patients receiving primary treatment elsewhere), 10 have died of metastases, and 3 are living, nine months, two years and three months, and 20 years and one month postoperatively. Of the 13 patients who died in the entire series, 9 died one year or less after seeking medical treatment. One patient (case 25), symptom-free for nine years after orchidectomy,

34 Sheetz, R. J., and Leddy, E. T.: Am. J. Roentgenol. **55**:754, 1946.

35. Sheetz and Leddy.[34] Bang, F.; Hamburger, C., and Nielsen, J.: Bull. Assoc. franc. p. l'etude du cancer **24**:418, 1935.

succumbed after two weeks of symptoms; autopsy revealed metastatic lesions in the lungs and the retroperitoneal and posterior mediastinal lymph nodes.

HISTOLOGICALLY BENIGN TERATOMA

Pathology.—The histologically benign teratoma may fall into one of three ill defined subtypes: (1) organized teratoma; (2) unorganized teratoma; (3) simplified teratoma. None of these contains recognizable histologically cancerous elements.

Organized teratoma is a rare complex tumor in which rudimentary organs are formed and may be so arranged as to resemble a malformed fetus. In unorganized teratoma neoplastic structures of bidermal or tridermal origin appear in disorderly arrangement with no abortive attempt at organ formation. Commonly observed are: solid nests of squamous epithelium; cysts lined by cells resembling respiratory, intestinal, renal, squamous or transitional epithelium; nests of myxomatous connective tissue, embryonic and mature cartilage, smooth muscle and glia. Foci resembling mixed tumors of salivary gland origin may be encountered. Simplified teratoma is a monodermal form. Its most common variety is the epidermoid cyst; considerably less frequent is the dermoid cyst.[16] Rarely chondroma, leiomyoma, rhabdomyoma, myxoma, lipoma or osteoma[9] is encountered.

Often it is found that histologically benign teratoma has metatastasized. The secondary deposits may show the structure of histologically cancerous teratoma or embryonal adenocarcinoma. The most satisfactory explanation for this phenomenon is that offered by Friedman and Moore,[16] namely, that the metastases are due to dissemination of a cancerous embryonal cell, the mother cell of both types of teratoma. Hence, the histologically benign tumor is composed entirely of mature descendants of a totipotential cell, whereas the cancerous variant contains immature offspring as well.

Endocrinology.—The endocrine aspects of the histologically benign teratoma have not been extensively investigated. Quantitative studies in small series have revealed little or no increase in excretion of gonadotropins in the urine.[31] In one qualitative determination[6] the excreted gonadotropin was the follicle-stimulating type.

Clinical Aspects.—No histologically benign teratoma was encountered in the present series. The percentage incidence reported elsewhere has varied from 4 to 9.[36]

Due to the scarcity of cases, knowledge of the clinical behavior of this type has been limited. The organized variety has been commonly

---

36. Chevassu.[10] Hamburger, Bang and Nielsen.[9] Friedman and Moore.[16] Hinman and Powell.[11d] Ferguson.[8]

regarded as slowly growing, usually congenital, and rarely undergoing cancerous change[37] while the unorganized and the simplified varieties have been viewed as having guarded prognoses.[38]

The observations of Friedman and Moore in their recent large series (68 cases) have modified the earlier concepts of prognosis. Their patients were for the most part between the ages of 18 and 38. No subclassification into organized, unorganized and simplified teratoma was attempted; however, it was stated that epidermoid and dermoid cysts constituted 16 per cent of the specimens and that the presence of highly specialized tissues and organs was rare. Although in most instances the period of observation was less than a year, the mortality rate was 15 per cent, while an additional 13 per cent of the patients were living with metastases. These figures were strikingly similar to those for cases of histologically cancerous teratoma (excluding cases of pure embryonal adenocarcinoma).

The metastases of histologically benign teratoma have been stated to be radioresistant.[11d]

COMMENT

Over 95 per cent of testicular neoplasms fall into two classes, seminoma and teratoma.

Seminoma occurs in males and females alike; in the former, it is frequently associated with cryptorchidism; in the latter, with pseudohermaphroditism. It apparently arises from the primordial (neuter) germ cell. Its striking histologic uniformity is due to the inert character of its cell, which appears incapable of differentiation. In males it occurs chiefly in the fourth decade and usually grows relatively slowly, metastasizing late and principally by the lymphatic route; it may, however, metastasize early and be rapidly fatal. It is highly radiosensitive and occasionally radiocurative. It tends to be associated with the presence of small quantities, or a total absence, of a follicle-stimulating substance, apparently of pituitary origin, in the urine.

Testicular teratoma is a distinctive tumor, differing in a stiking fashion not only from extragenital teratoma but from ovarian teratoma as well. It apparently arises from a totipotent sex cell or from one of the embryonic predecessors of this cell. Histologically, it may run the gamut from a highly undifferentiated embryonal neoplasm to a mature teratoma showing no microscopic evidence of cancer. It frequently contains trophoblastic elements, and rarely appears as pure chorionic carcinoma. It occurs chiefly in the third decade and characteristically grows rapidly and metastasizes early, commonly by way of the blood stream;

37. Ewing.[9b] Chevassu.[10] Hinman.[2a]

38. Chevassu.[10] Hinman.[2a]

it may, however, be of relatively slow progression. It is rarely radiosensitive, and almost never radiocurative. It may be associated with the presence of a follicle-stimulating substance in the urine, but more typically, depending on the amount of trophoblastic tissue which it contains, it may secrete variable quantities of chorionic gonadotropin and perhaps progesterone and estrogens.

Occasionally seminoma and teratoma grow side by side; occasionally they become intimately mixed.

SUMMARY

A clinicopathologic analysis is made of 33 cases of testicular neoplasms common in adults.

Important contributions in the literature are briefly reviewed.

Testicular tumors are divided into two large types, seminoma and teratoma. The clinical, biologic and pathologic features of the two types are contrasted.

VARICES, CONGENITAL SYPHILIS, TUBERCULOSIS AND SECONDARY TUMORS OF THE ESOPHAGUS

Histologic Observations

JAN F. MAJERANOWSKI, M.D.

OTTAWA, CANADA

THE ESOPHAGUS is not usually subjected to routine histologic examination at autopsy. For this reason a study of a large number of esophagi, from selected cases, was undertaken. The specimens were obtained from the department of pathology of the University of Edinburgh and the Royal Infirmary, Edinburgh. It is the purpose of this communication to describe and comment on certain of the lesions encountered in the study of this material.

ESOPHAGEAL VARICES

The lower segment of the esophagus was examined in its entire circumference by means of serial sections in 12 cases of cirrhosis of the liver in which there was a history of repeated hematemesis. In all these cases autopsy revealed visible varicosities of the esophagus, but gross evidence of mucosal erosion or venous rupture was completely absent.

The question of the mode of origin of the esophageal varices is intimately connected with the arrangement of veins in the lower part of the esophagus. The lamina propria of the mucosa is especially rich in venous supply, and the veins are situated in a loose connective tissue. It is easy to see how readily they can be distended on increase of the blood pressure. Figure 1*A* illustrates distention of veins. The degree of dilation is not extreme. Frequently one encounters sinus-like dilatations of far greater diameter.

As has been pointed out, in all of the cases in this small series there had been repeated hematemesis during life; yet at autopsy gross examination failed to reveal possible sources of hemorrhage in the esophagus. However, in 5 of the 12 cases a lesion was found on microscopic examination which would appear to account for hemorrhage and yet remain invisible to the naked eye at autopsy. In figure 1*B* such a lesion is illustrated. Dilated veins are seen in the subepithelial tissues of the esophagus. The overlying epithelium shows two clefts or fissures.

From the Department of Pathology, University of Ottawa.

This article was prepared from the material presented in a thesis for the Degree of Doctor of Medicine at the Polish School of Medicine of the University of Edinburgh.

Both communicate with venous spaces and one, on the left side of the photomicrograph, contains erythrocytes. It is suggested that lesions of this type may be the concealed source of hemorrhage.

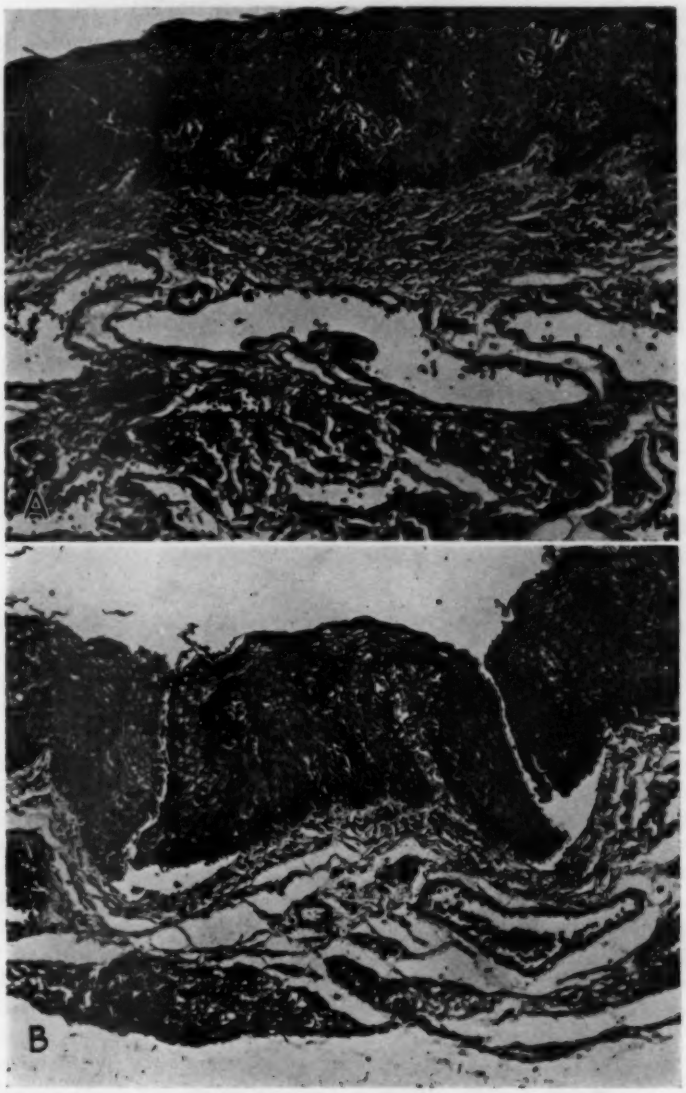

Fig. 1.—*A*, photomicrograph of esophagus showing dilated venous sinuses in the lamina propria of the mucosa. × 100.

B, photomicrograph of esophageal epithelium showing two fissures which communicate with venous spaces in the lamina propria of the mucosa. The fissure on the left contains erythrocytes. × 100.

CONGENITAL SYPHILIS

Syphilitic esophageal lesions are rare but may appear in the late, tertiary stages of the disease. Abel[1] stated that 0.1 to 0.2 per cent of syphilitic patients have esophageal complaints. Usually the lesions are gummatous in type and lead to various forms of ulceration and cicatricial stricture. Congenital syphilis, according to Abel, is still more rare.

Seven cases of infantile congenital syphilis were examined in the present study. These were serologically proved to be cases of syphilis, and in all spirochetes were demonstrated in tissues. In none were there visible esophageal lesions, but in 4 of the 7 cases histologic study revealed significant inflammatory changes interpreted as syphilitic. Figure 2*A* illustrates a typical lesion. The lamina propria of the mucosa is widened, the fibers of the connective tissue are swollen, and there are collections of inflammatory cells disposed in collar-like fashion about small blood vessels. Figure 2*B* shows the same lesion under higher magnification. Lymphocytes, plasma cells and larger mononuclear cells can be identified. The involvement was confined to the lower segment in 3 cases. In 1 case there were diffuse patchy infiltrations throughout the entire length of the esophagus.

TUBERCULOSIS

Tuberculosis of the esophagus is extremely rare. Guggenheim, Rosenberg and Laff[2] stated that, of all organs subject to tuberculous infection, the esophagus is least often affected.

Two cases of esophageal tuberculosis are briefly reported:

CASE 1.—The patient was a man aged 56. The esophagus showed five areas of ulceration. Three were grouped together about 12 cm. below the level of the larynx. These were oval, with an average long diameter of 1 cm. Seven centimeters lower were two slightly larger, deeper ulcers, having altered blood at their bases. In the involved areas the esophagus was adherent to adjacent tissues. A small caseous lymph node was discovered beneath the floor of one of the lower ulcers. One hilar lymph node showed a similar caseous focus. Otherwise a complete autopsy failed to reveal any healed or active tuberculous lesion. Death was due to advanced bronchopneumonia. Histologic study of the ulcers showed their bases to be formed of active tuberculous granulation tissue and caseous material. Deeper in the esophageal wall were conglomerates of tubercles and numerous small caseous foci, while the space between the esophagus and the trachea was occupied by scar tissue containing carbon particles. One of the lower ulcers had a completely eroded vessel in its base; the arterial lumen contained partly organized thrombotic material. Figure 3*A* shows a portion of one ulcer. Its base is composed of caseous material. Tuberculous granulation tissue is seen, and at the right hand side of the photomicrograph there are two Langhans giant cells. Figure 3*B* shows a section taken from the esophageal wall close to one of the ulcers and shows what is evidently a preulcerative stage. Just under the elevated, but still intact, epithelium is an enlarged lymph follicle in which is a small focus made up of epithelioid cells.

CASE 2.—The patient was a man aged 62. In the esophagus was an area of ulceration with caseous base and undermined edges. Between the esophagus and the trachea

1. Abel, L.: Lancet **2**:441, 1926.
2. Guggenheim, A.; Rotenberg, L.; and Laff, H.: Am. Rev. Tuberc. **46**:577, 1942.

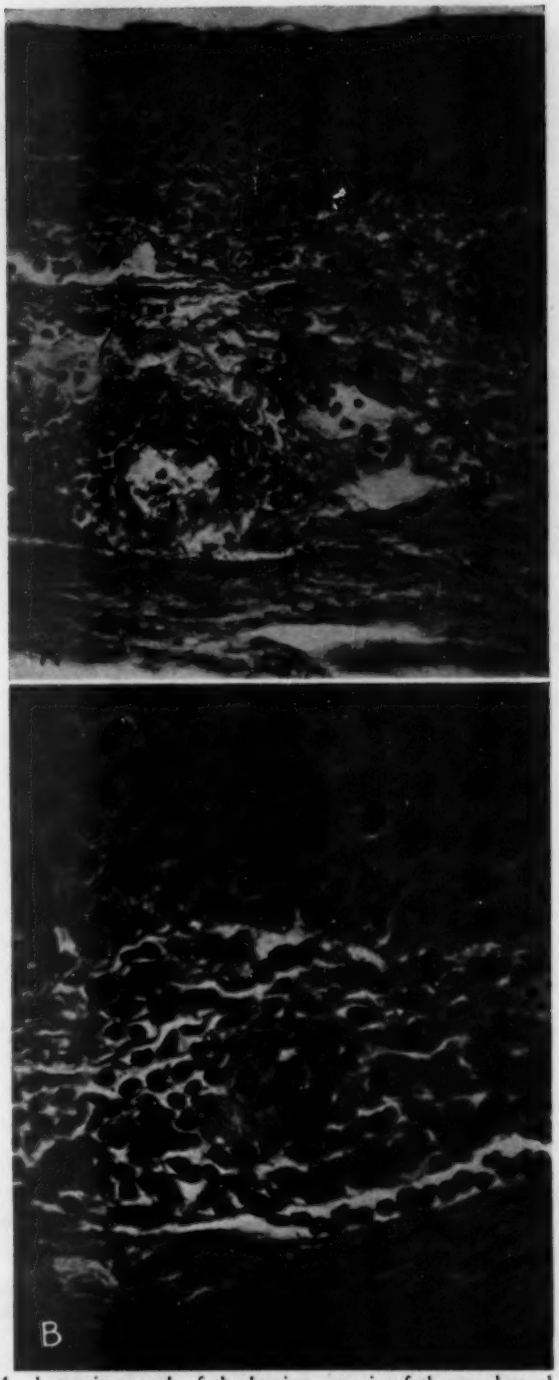

Fig. 2.—*A*, photomicrograph of the lamina propria of the esophageal mucosa showing an infiltration of mononuclear inflammatory cells, some of which are in perivascular arrangement. × 240.

B, photomicrograph of the lesion shown in *A* under higher magnification. Lymphocytes, plasma cells and histiocytes can be seen. × 500.

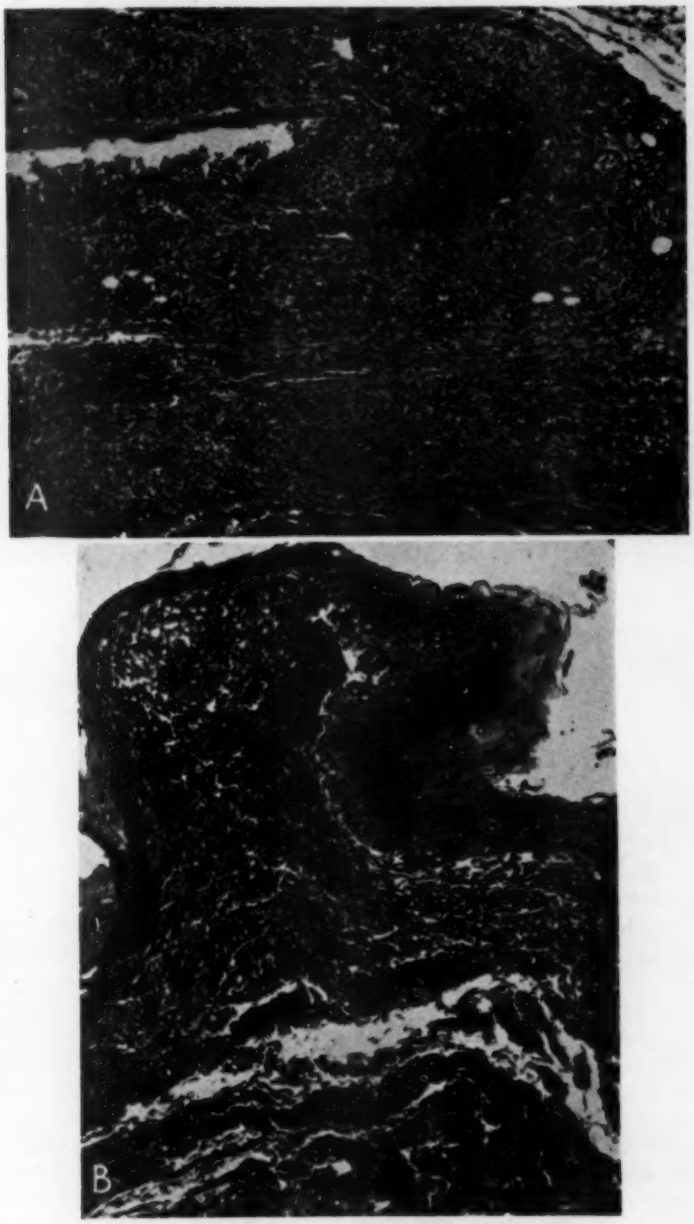

Fig. 3.—*A*, photomicrograph of esophageal wall showing tuberculous granulation tissue. Two Langhans giant cells are seen in the right lower corner of the photomicrograph. × 100.

B, photomicrograph of esophageal wall. In the left upper corner, in the lamina propria of the mucosa a small collection of epithelioid cells is visible. The epithelium is thinned out but still intact. × 110.

at the level of ulceration was a mass of fibrocaseous nodes. A similar mass of caseous mesenteric lymph nodes was present, and miliary tubercles were found in the liver, the spleen, the kidneys, the cecum and the rectum. Histologically, the ulcer revealed an appearance similar to that described in the first case.

It will be seen that in the first case the two lower ulcers were in continuity with a tuberculous lymph node, while the other ulcers may have developed from hematogenous seeding of bacilli from the first lesion or from the caseous hilar node. In the second case again, either direct spread or hematogenous dissemination may have been the cause of esophageal involvement.

SECONDARY TUMORS

In 14 cases of diffuse metastatic spread of cancer, the lamina propria of the esophageal mucosa was discovered to be involved in 6. The

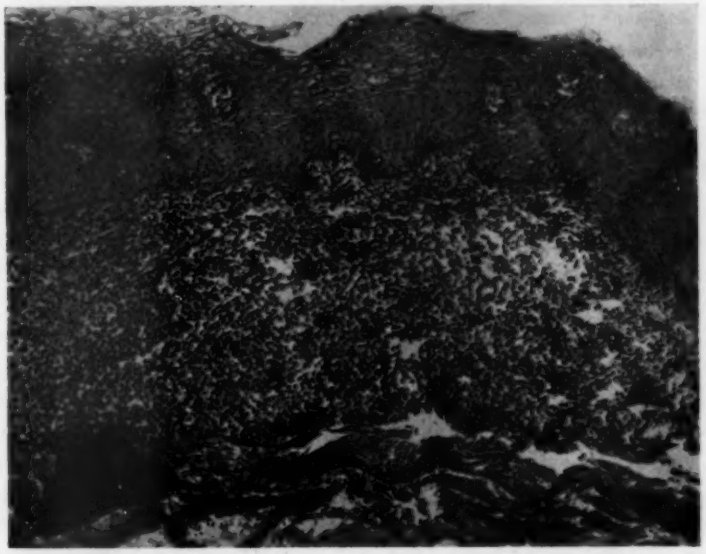

Fig. 4.—Photomicrograph of the lamina propria of the esophageal mucosa showing secondary reticulum cell sarcoma. × 80.

esophagus showed no changes to the naked eye in any of these cases. In 2 of them the submucosa and the circular muscle of the tunica muscularis were also involved. The tumor cells were arranged in groups and scattered foci. Figure 4 shows infiltration of the lamina propria of the esophageal mucosa by reticulum cell sarcoma. At autopsy, gross examination revealed involvement of the liver, the kidneys, the mesenteric lymph nodes, the lungs and the skin.

COMMENT

The explanation of hemorrhage of esophageal varices usually advanced is that distended veins exert pressure on the overlying epithelium, with resultant erosion, ulceration and eventual rupture of the

exposed wall of the vein. Yet only rarely in cases in which death has been due to massive hemorrhage, can one at autopsy, even with injection technics, locate the exact point of bleeding or find a damaged vein. This is due mainly to the fact that after death the blood drains into the larger vessels, leaving the esophageal veins collapsed and bloodless. However, in 5 of the 12 cases of cirrhosis of the liver, in this series, microscopic fissures were discovered in the epithelium, communicating with venous spaces. Some fissures contained erythrocytes. All the patients had suffered from repeated hematemesis during life; yet at autopsy the esophagus presented no gross changes. There was no evidence of mucosal ulceration or of rupture of a varix. It is suggested that these epithelial fissures may be the source of hemorrhage. It seems probable that epithelium devitalized by the pressure of underlying varicosities and abraded, further, by food particles may be prone to the development of such lesions. During life, when the esophagus is the site of peristaltic waves and when the veins are distended with blood, such fissures may gape to a considerable extent, and if they communicate with veins, they may form a channel for hemorrhage, a channel which remains concealed at autopsy.

When the esophagus is examined in cases of congenital syphilis, it is frequently shown to be affected. In view of the widespread visceral involvement, this is perhaps not unexpected. Yet it has largely escaped comment in the medical literature on the subject. Congenital syphilitic esophagitis may be overlooked at autopsy unless histologic examination is carried out, since to the naked eye it gives no evidence of its presence.

Tuberculous esophagitis is admittedly rare. Flexner,[3] in 1893, was the first to classify tuberculous lesions of the esophagus pathogenetically. He considered that these lesions could be due to infection extending from neighboring structures (caseous bronchial or mediastinal lymph nodes, an ulcerated tuberculous pharynx or vertebrae involved in tuberculosis) or to bacilli brought from distant organs (by blood stream or by infected sputum). He stressed the importance of local predisposing lesions (stricture, neoplasm, thrush). It seems clear that esophageal tuberculosis is almost always secondary to some other focus of infection. It is true that Torek[4] has reported a case without clinical evidence of active tuberculosis elsewhere, but the case lacks autopsy confirmation. Cone,[5] continuing Flexner's work, analyzed 48 cases of esophageal tuberculosis, compiled from various sources, and came to the conclusion that in 24 infection had spread from the surrounding tissues, in 4 it had a hematogenous origin. in 15 it was due to bacilli inoculated from infected sputum, while in 5 tuberculosis developed from preexisting local lesions. She does not specify the mode of infection in the last 5 cases. The 2 cases of esophageal tuberculosis en-

3. Flexner, S.: Bull. Johns Hopkins Hosp. **4**:4, 1893.
4. Torek, F.: Ann. Surg. **94**:794, 1931.
5. Cone, C.: Bull. Johns Hopkins Hosp. **8**:229, 1895.

countered in the present series demonstrate that it is often difficult to decide whether infection has spread directly from neighboring tuberculous lymph nodes or been carried via the blood stream.

It is evident from this study that the loosely arranged tissue between the epithelium and the muscularis mucosae of the esophagus—in other words, the lamina propria of the mucosa—is susceptible to infection with hematogenous tubercle bacilli and Treponema pallidum and also to secondary deposition of cancer cells. This feature of the lamina propria of the mucosa may be partially explained on the ground of its marked vascularity.

SUMMARY

Certain lesions encountered in a study of a large number of esophagi from selected cases have been presented. Epithelial fissures have been described, which may form a channel for a concealed source of hemorrhage of esophageal varices. The susceptibility of the lamina propria of the mucosa to congenital syphilis, tuberculosis and secondary neoplastic disease has been noted. It is suggested that the marked vascularity of this tissue explains to a certain extent its susceptibility to involvement from hematogenous spread of these diseases.

PULMONARY EMBOLI COMPOSED OF CONTENTS OF AMNIOTIC FLUID

ELMER R. JENNINGS, M.D.

AND

BERT E. STOFER, M.D.

DETROIT

IN THE SEVEN YEARS which have elapsed since the first description of maternal pulmonary embolism caused by particulate material contained in amniotic fluid, only three reports of this type have been published. We have recently encountered a case which, together with the 14 previously reported, makes a sufficient number so that a review of the series is thought to be timely.

Steiner and Lushbaugh[1] reported the first 8 cases in which this type of pulmonary embolism was observed. These cases were encountered in a fifteen year period at the Chicago Lying-in Hospital, during which death from this cause occurred once in each 8,000 deliveries. In the experience of these authors it was the most common cause of maternal death occurring during labor or the first ten hours of the puerperium. The outstanding clinical feature was a shocklike state that developed rapidly either during labor or shortly after its termination. The most common clinical diagnosis was obstetric shock. Other diagnoses were rupture of the uterus, hemorrhage, abruptio placentae, acute left ventricular failure and toxemia without convulsions. These workers were able to reproduce the clinical and pathologic aspects of this disease in rabbits and dogs by injecting saline suspensions of meconium or amniotic fluid containing squamae and vernix caseosa. Amniotic fluid which had been filtered to remove all particulate matter did not produce the disease in the experimental animals.

In 1942 Lushbaugh and Steiner[2] reported 2 more cases in which additional features were observed. One patient succumbed to rupture of the uterus seven days after delivery, and lanugo hairs were found in the pulmonary emboli. In the lungs foreign body granulomas were also encountered. These were similar to those observed in the healing stage

---

From the Department of Pathology, Wayne University College of Medicine and City of Detroit Receiving Hospital.

1. Steiner, P. E., and Lushbaugh, C. C.: J.A.M.A. **117**:1245, 1941.
2. Lushbaugh, C. C., and Steiner, P. E.: Am. J. Obst. & Gynec. **43**:833, 1942.

of the disease in experimental animals which was produced by the intravenous injection of a sublethal dose of the particulate matter of amniotic fluid. In the second case no uterine contractions had occurred, but embolism resulted from opening of the placental sinuses while laparotrachelotomy was being done for placenta previa.

Hemmings[3] added a case in 1947, in which no new features were observed. Gross and Benz[4] have provided the most recent communication describing 3 cases. These occurred in one year and represented all the maternal deaths occurring in that year in a service in which 1,200

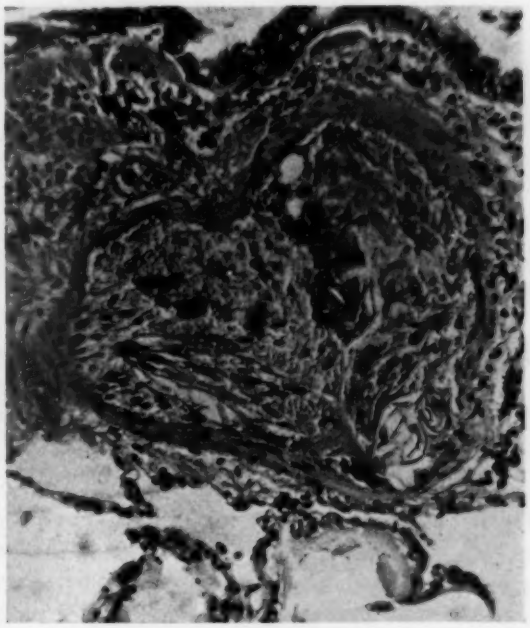

Fig. 1.—Lung showing small peribronchial artery almost completely occluded by an embolus consisting of mucus, squamae and a few leukocytes. Hematoxylin and eosin stain; × 150.

deliveries were made. They devised a technic by which a rapid diagnosis was made. It consists in centrifuging blood aspirated from the inferior vena cava or the right side of the heart and noting a layer of pale material between the buffy coat and the serum. When smears and sections were made of this material, mucus, squamae, leukocytes and finely granular material were seen. This test may be done even though a necropsy is not performed.

3. Hemmings, C. T.: Am. J. Obst. & Gynec. **53**:303, 1947.
4. Gross, P., and Benz, E. J.: Surg. Gynec. & Obst. **85**:315, 1947.

REPORT OF A CASE

D. V., a 28 year old white woman, a primigravida, was admitted to the Detroit Receiving Hospital at 8:35 a.m., Nov. 4, 1947. She had noted labor pains since 6 a.m. Her last menstrual period was April 20, 1947. The fetal head had engaged, and the position of the fetus appeared to be left occipitoanterior. Moderately severe secondary anemia was present. Labor continued normally, so that the crevix was dilated to 5 cm. At this time the membranes ruptured spontaneously. It was noticed that the amniotic fluid contained blood. She was given 0.32 mg. of scopolamine hydrobromide and 100 mg. of meperidine hydrochloride ("demerol hydrochloride") and taken to the delivery room at 3 p.m. There she suddenly became dyspneic and cyanotic; a blood pressure reading was not obtainable. She died at 3:10 p.m. Since the fetal heart sounds were still

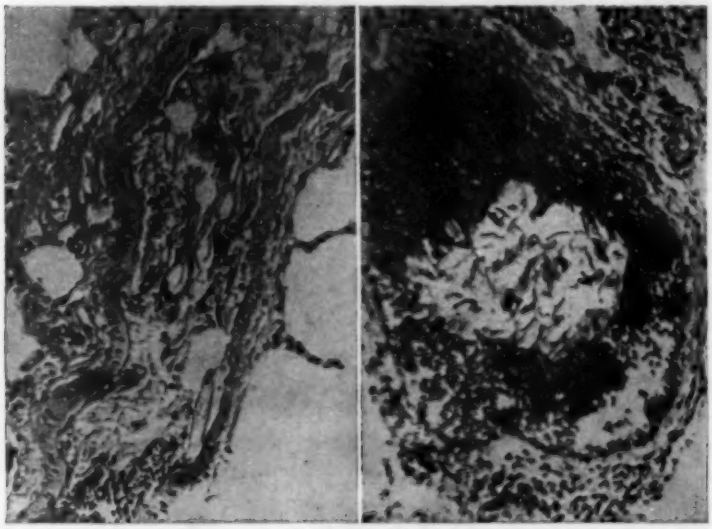

Fig. 2—Left: Lung showing an arteriole in longitudinal sections, which is partially occluded by an embolus of mucus, squamae and lanugo hair. Hematoxylin and eosin stain; × 150.

Right: Vein of an ovarian plexus containing an embolus of squamae. Hematoxylin and eosin stain; × 150.

audible, a cesarean section was made. Slight intrauterine hemorrhage and marginal implantation of the placenta were noted at operation. A living boy was obtained, who had respiratory difficulty and died at 9 a.m. the following day.

Pathologic Considerations.—At necropsy, three hours after the death of the mother, the positive abnormalities consisted only of atonic uterus, hyperemic spleen, mild bilateral hydroureter and moderate atelectasis of the left lung. There was no pulmonary edema; the combined weight of the lungs was 455 Gm. The placenta was not available for study. On histologic examination, the blood in many of the small pulmonary arteries, arterioles and capillaries was almost entirely replaced by epithelial squamae, strands of mucus, leukocytes and granular debris. In one vessel a pale yellow cylindric object was seen, which was interpreted as a lanugo hair. Squamae were present in the veins of the ovarian plexus. The infant was not examined post mortem.

COMMENT

Table 1 contains some of the pertinent data collected in the 15 cases which have been reported. The average age of the patients was 33 years, and all but 2 were multiparas. When Steiner and Lushbaugh analyzed the first 8 cases, they concluded that the disease occurred in elderly multiparas. This appears to be generally true. The duration of pregnancy varied from twenty-eight to forty-eight weeks, with an average of forty weeks. The total duration of labor varied from four to fifty hours, with an average of twenty-four hours for those cases in which this information was available. In about two thirds of the patients the uterine contractions were strong or tetanoid. By some authors[1] this has been considered an important factor in the production of the disease. The average weight of the infant in 6 previously reported cases was 4.4 Kg., which supports the opinion of Steiner and Lushbaugh that overweight of the fetus is a causative factor. While this may be generally true, it is not an invariable finding, as is illustrated by the case reported here. The exact role of death of the infant in the production of amniotic embolism is not clear. From table 1 it is evident, however, that in only half the cases did the infant survive the death of the mother. The time relations between rupture of membranes, onset of shock, delivery and maternal death are set forth in table 2. The interval between rupture of the membranes and onset of symptoms of "shock" varied from thirty minutes to seventy-two hours. The compiled data indicate that the onset of symptoms occurred most often near the termination of the second stage of labor and that the average elapsed time between the onset of symptoms and death was three hours. It was during this interval that delivery most frequently took place. These time relationships suggest that when delivery is imminent, some change occurs which permits amniotic fluid to enter the maternal circulation. An observation not tabulated is the presence of meconium or of blood in the amniotic fluid, the former having been reported four times and the latter three times.

The normal sequence of events in this type of maternal death is rupture of the membranes, shock, delivery and death. The amniotic fluid probably gains access to the maternal circulation in one of three ways: There may be a tear in the fetal side of the placenta; in some cases premature placental separation may open the route; when the onset of shock follows delivery of the placenta, the most likely pathway is through the site of placental attachment. As yet, however, no direct observation of either of the first two means of access has been described. In 11 of the 15 cases strong or tetanoid contractions of the uterus were exhibited, and it is known that such contractions are at times the result of premature separation of the placenta. This feature suggests that premature separation of the placenta may operate in the majority of cases.

Any theory of the pathogenesis must take into consideration those cases in which the time intervening between rupture of the membranes and onset of shock is prolonged. Since experimentally the shock began soon after the injection of amniotic fluid or meconium suspended in saline solution, one might assume in cases of prolonged interval that the fluid

TABLE 1.—*Clinical Features of 15 Cases*

Case	Author	Date	Author's Case No.	Parity	Age	Duration of Pregnancy Weeks	Duration of Labor Hours	Characteristics of Contractions	Weight of Fetus, Gm.	Condition of Fetus
1	Steiner and Lushbaugh[1]	1941	1	30	2	38	48	Strong		Macerated
2	Steiner and Lushbaugh	1941	2	26	1	42	32	Strong	3510	Living
3	Steiner and Lushbaugh	1941	3	28	4			Strong	4086	Living
4	Steiner and Lushbaugh	1941	4	37	3			Strong	4604	Dead
5	Steiner and Lushbaugh	1941	5	42	8				—	Living
6	Steiner and Lushbaugh	1941	6	34	2	48		Strong	5180	Living
7	Steiner and Lushbaugh	1941	7	33	4			Strong	5568	Living
8	Steiner and Lushbaugh	1941	8	25	2		50	Strong	—	Macerated
9*	Lushbaugh and Steiner[2]	1942	1	35	2		4	Not strong	—	Dead
10*	Lushbaugh and Steiner	1942	2	38	2		Op.	0	—	Living
11	Hemmings[3]	1947	1	33	2	42	18	Strong	3500	Dead
12	Gross and Benz[4]	1947	1	25	2	43	12.5	Strong	—	—
13	Gross and Benz	1947	2	42	3	39	37	Strong	—	Dead
14	Gross and Benz	1947	3	35	5	36	8	Not unusual	—	Living
15	Jennings and Stofer	1948	1	28	1	28	9	Not unusual	—	Living Premature

*The patient did not die of pulmonary embolism.

TABLE 2.—*Time Relationships Between Rupture of Membranes, Shock, Delivery and Death**

Case	Time Between Rupture of Membranes and Onset of Shock, Hr.	Time of Onset of Shock in Relation to Delivery, Hr.	Time Between Onset of Shock and Death, Hr.	Time Between Delivery and Death
1		1.25 before	3.75	2.5
2	19	Undelivered	1	Undelivered
3		At delivery	8†	8
4		9.5 before	11.5(?)	1.5
5		0.1 after	1	1
6		3.0 before	5	2
7		At delivery	2	2
8		Undelivered	2	Undelivered
9†				7 days
10†		At operation		Death occurred after operation
11	72	5.0 before	5.5	0.5
12	3	0.5 before	0.6	0.1
13	35†	At delivery	1	1
14	0.5	0.3 after	1	1.3
15	0.5	Undelivered	0.2	Undelivered

*All times are recorded in hours.
†The patient did not die of pulmonary embolism.

had been incarcerated in the uterine cavity until one of the three routes is made available. However, fluid trapped in this manner has not been described.

The placenta of the delivered patient with amniotic pulmonary embolism should be carefully inspected to establish the point

of rupture. In the case of the undelivered patient the necropsy should include an attempt to establish, in addition to the point of placental rupture, the amount of fluid in the uterine cavity and the degree of placental separation.

SUMMARY

A case of maternal pulmonary embolism caused by blood stream dispersions of the contents of the amniotic fluid in the twenty-eighth week of gestation is reported.

The medical literature dealing with this type of maternal death is reviewed, and the 15 reported cases are tabulated.

The establishment of embolism of this type as a well recognized entity is important. It is urged that more cases be reported and that this cause of death be considered in all cases of so-called obstetric shock. The value of rapid diagnosis by examination of the circulating blood should be determined.

MAST CELLS

Their Distribution in Various Human Tissues

JOSEPH JANES, M.D.
AND
JOHN R. McDONALD, M.D.
ROCHESTER, MINN.

IT HAS BEEN a common clinical observation on our part as well as on that of a host of others that in closed injuries of joints the hemarthrosis is often fluid. That this phenomenon is not limited to joints is attested by the fact that one has often aspirated blood from the pleural cavity and from subcutaneous tissue spaces. The question which comes to mind is, "What keeps the blood from clotting in these sites?" Best and Taylor[1] stated that under normal circumstances the blood remains fluid in the vessels, most probably because thromboplastin is present in circulating blood in only very small amounts, and as a consequence the active enzyme is not produced and fibrin is not formed. These authors did not mention what keeps the blood fluid in the locations cited in the first two sentences of this paragraph. They did state that heparin is present only in minute amounts in the circulation and at any rate is not responsible for maintaining the fluidity of blood in the human body.

Masella[2] has concluded from an experimental study that the synovia possibly has an inhibiting action on the retraction of the clot. He also expressed the belief that the hemarthrosis may remain fluid because the synovia fixes the salts of calcium. The latter view is difficult to reconcile with the statement of Best and Taylor[1] that in hypoparathyroidism, in which the serum calcium may be depressed to less than half the normal value, the coagulation time is not lengthened. According to Kling[3], fibrinogen is low in chronic effusions, and as a result coagulation is either absent or delayed and incomplete in chronic effusions of joints. This does not account for the fluid hemarthrosis of

---

From the Section on Orthopedic Surgery and the Section on Surgical Pathology, Mayo Clinic.

1. Best, C. H., and Taylor, N. B.: The Physiological Basis of Medical Practice, Baltimore, Williams & Wilkins Company, 1943, pp. 147-148.

2. Masella, T.: Sperimentale, Arch. di biol. **92**:339, 1938.

3. Kling, D. H.: The Synovial Membrane and the Synovial Fluid, with Special Reference to Arthritis and Injuries of the Joints, Los Angeles, Medical Press, 1938.

acute trauma. Riedel[4] in 1880 and Jaffee[4] in 1898 stated that the blood in hemarthrosis coagulates and is redissolved by the admixture of synovial fluid after twenty-four hours. This view does not explain the fluid hemarthrosis which may be aspirated within the first twenty-four hours after injury. Kling[3] went on to state that in vivo the synovial lining and synovial fluid have an anticoagulative effect. From his own studies he has demonstrated that in traumatic effusions of human joints most of the hematomas were liquid, even when aspiration was performed only a few hours after injury. Only in large hematomas were some coagulums recovered, and he attributed these to extensive tearing of the capsule with coagulation having originated in the extra-articular structures. He did not mention the specific factor which inhibits coagulation, although he did state that the irritation provoked by the hemarthrosis leads to an increased production of mucin by the synovial membrane. Cherry and Ghormley[5] have shown that the cells lining the synovial membrane contain a high concentration of mucin and suggested that these cells are the source of the mucin in the synovial membrane.

Holmgren and Wilander[6] expressed the belief that heparin is the physiologic anticoagulant of the body and that this substance is produced by the mast cells. They pointed out that the metachromatic substance of mast cells is identical with heparin. They, along with Jorpes and Bergstrom[7], have done some excellent work on the subject and their experimental work would seem to verify their convictions. To recapitulate, they postulated that the widely disseminated cell system which is formed by Ehrlich's mast cells can be thought of as the site of the formation and secretion of an anticoagulant substance (heparin).

Because of this interesting postulation of the Swedish investigators, we determined to make a study of the occurrence of the mast cells in various human tissues in various pathologic states, paying particular attention to their occurrence in the tissues entering into the formation of joints. The problem, therefore, herein presented is of a histologic nature, although reference will be made to a crude chemical experiment which tends to support the interpretation that heparin is the substance which is at least in part responsible for the fluidity of the blood in hemarthrosis.

The literature written in English on the distribution of mast cells which was reviewed did not seem to cover the subject matter of this

---

4. Cited by Kling,[3] p. 174.
5. Cherry, J. H., and Ghormley, R. K.: J. Bone & Joint Surg. **20**:48, 1938.
6. Holmgren, H., and Wilander, O.: Ztschr. f. mikr.-anat. Forsch. **42**:242, 1937.
7. Jorpes, E., and Bergstrom, S.: Ztschr. f. physiol. Chem. **244**:253, 1936.

paper completely, although Staemmler[8] (German) in 1921 studied the occurrence of mast cells in most of the human organs under normal as well as under pathologic conditions. He concluded that mast cells were found in fibrillar connective tissue and that they may be thought of as being one-celled glandular organs of the connective tissue.

HISTORICAL BACKGROUND, CYTOLOGY AND HABITAT

According to Boyd,[9] the mast cell is a cell with coarse basophilic granules in the cytoplasm and an indented nucleus of the neutrophils. It is the basophil leukocyte of the blood, present normally in small numbers, and it is also found in the tissues. These cells are observed in mild, subacute inflammations, but they show a marked tendency to disintegrate, so that the granules alone may be seen. Mast cells stain metachromatically with toluidine blue. As Jorpes and Bergstrom[7] have pointed out, tissues which are rich in heparin stain in a similar manner. Examples are the subintimal tissue of blood vessels and subpleural and subperitoneal connective tissue. It is reasonable to suggest that mast cells are concerned with the production of heparin. As stated by Michels[10], the mast cell is connected with the early knowledge of connective tissue. Therefore, although they did not name them, von Recklinghausen in 1863, Kuhne in 1864, Kolliker in 1867, Friedlander in 1867, Flemming in 1867, Cohnheim in 1869, Rollett in 1871 and Schobl in 1871 may be considered together as the discoverers of the mast cells. Waldeyer [11] described mast cells in 1875 but included them with the plasma cells. He noted their perivascular habitat.

In 1877 Ehrlich[12] introduced the term "mast cell" and showed that mast cells could be metachromatically stained with dahlia. He recognized them as a variety of connective tissue cells. Believing that they had something to do with the nutrition of tissue, he named them "mast cells," "mast" being the German adjective meaning "sleek, fat, well fed." This term has been considered inappropriate in the light of subsequent studies.

Embryologically, the mast cell is believed to arise from the mesenchyme. If the substance called volutin, which is present in plants (algae, mushrooms) and protozoa, can be proved to be identical with the mast granules, then the gamma or mast granules would be represented in all forms of life. In connection with the cytologic characteristics, the

8. Staemmler. M.: Frankfurt. Ztschr. f. Path. **25**:391. 1921.

9. Boyd, W.: A Textbook of Pathology: An Introduction to Medicine, ed. 4, Philadelphia. Lea & Febiger, 1943, p. 110.

10. Michels, N. A.: The Mast Cells, in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 1, pp. 235-372.

11. Cited by Michels.[10]

12. Ehrlich, P.: Arch. f. mikr. Anat. **13**:263, 1877.

habitat and the function of mast cells, we quote extensively from the excellent monograph by Michels.[10] In man and most mammals the tissue mast cell is prevailingly polymorphous in cytoplasmic contour. The nucleus is relatively small as compared with the cell volume. As the cytoplasm stains little if at all, the outline of the cell body is to be taken from the aggregate granules. As the cell may be ruptured through technical operation, the granules may be found scattered at varying distances from the cell body (fig. 1). Accordingly, the mast cell may be found to be oval, piriform, spindle or star shaped. Harris[13] stated that, whereas oval and oblong forms vary in diameter from 3.5 to 14 microns

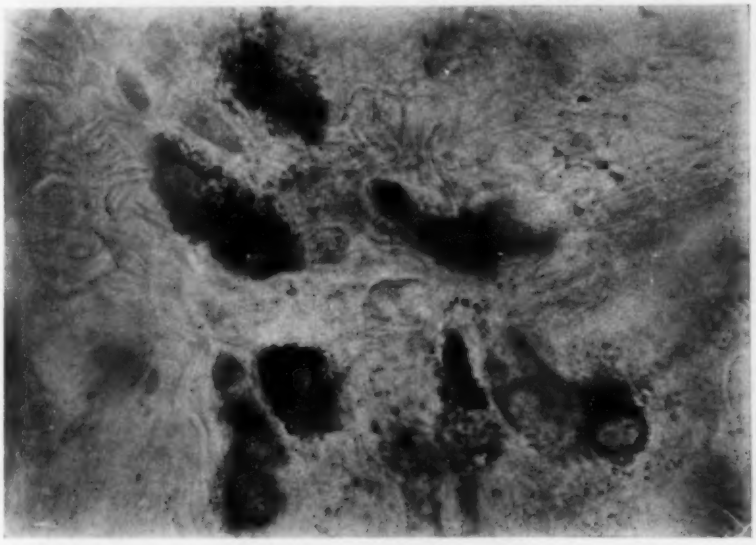

Fig. 1—Mast cells (x 800).

in instances, they attain a length of 28 microns. The mean diameter of the nucleus is given as 4 microns (man). The nucleus is usually round or oval, less seldom longitudinally stretched, indented or kidney shaped.

Changes in the morphologic characteristics of tissue mast cells may be due to various factors, intrinsic or extrinsic. Among the former may be listed (1) genetic origin, (2) ameboid movement and (3) physiologic conditions of the cell; among the latter (1) conditions of the environment or medium and (2) fixation, embedding and staining. The granules of the human tissue mast cell are usually spherical, occasionally oval, their mean diameter varying from 0.2 to 0.4 micron. The granules are often so numerous as to obliterate all view of the nucleus. Scattering of the granules of mast cells outside the cell bodies, many of which

are relatively intact, has been noted by many investigators. This apparently is due to a mechanical rupture of the cell bodies, the scattering of the granules in most cases being caused by the microtome knife. The chief characteristics of the mast cell granules are their basic metachromatic staining reaction and their solubility properties. The mast granules are soluble in water, and in order that they may be preserved, tissue must be fixed in 50 per cent alcohol and stained in alcoholic thionine. Holmgren and Wilander[6] advised using 10 per cent basic lead acetate as a fixative to render the metachromatic substance of the cells insoluble. The following basic dyes stain mast granules metachromatically: thionine, toluidine blue, methylene blue, methylene violet, cresyl violet, neutral ethyl violet, brilliant cresyl blue, amethyst, acridine red, neutral red, pyronine, safranine and azure. The blue dyes give a reddish, the red dyes a yellowish, tone to the granules (fig. 2).

Tissue mast cells are said to occur in all regions of the body. This statement was not confirmed in this study (see later comment concerning this). They are associated with connective tissue, and their abundance is usually dependent on the amount of connective tissue present.

Large numbers of tissue mast cells are present in the various layers of the corium, in the superficial and deep fascia and about hair follicles. Large groups of them have been found in the corium and subepithelial connective tissue in experimental tar cancers. Many mast cells are present in splenic lymph nodes, hemolymph nodes and tonsils.

Histogenous mast cells are fairly abundant in the bone marrow of mammals. Maximal numbers of mast cells are present in serous membranes. However, the hernial sacs examined in this study failed to reveal any large number of them. They are said to be present in the nervous system. Mast cells are present throughout the digestive tract in the connective tissue of the muscularis, in the serosal layer and especially in the submucosa and mucosa.

Certain organs are said to have a high quota of mast cells: *(a)* thymus; *(b)* uterus—in the connective tissue of the myometrium; *(c)* mamma—both in the resting and in the active phases. Staemmler[8] found them to be extremely sparse in the uterine mucosa. In our study, no mast cells were observed in the uterine mucosa in its various phases.

Certain tissues are devoid of histogenous mast cells. Cartilage and bone do not have them. Their complete absence in scar tissue is well known. They are present in diminished numbers in tissue involved in acute inflammation.

The tendency for mast cells to aline themselves along blood vessels (fig. 3*A*), especially capillaries, is recognized by all observers. It is an attractive hypothesis to postulate that they contribute some substance

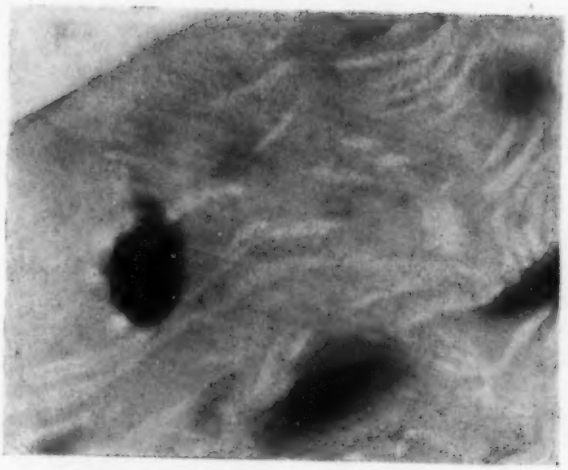

Fig. 2.—Mast cell in color (Terry's polychrome methylene blue; x 800).

to the blood. That mast cells may be disposed in densely packed clusters has been noted by many investigators, including ourselves.

Since this study was begun, it has come to our attention that Holmgren has done work to show that mast cells are bound to the connective tissue in various organs and in proportions which to a

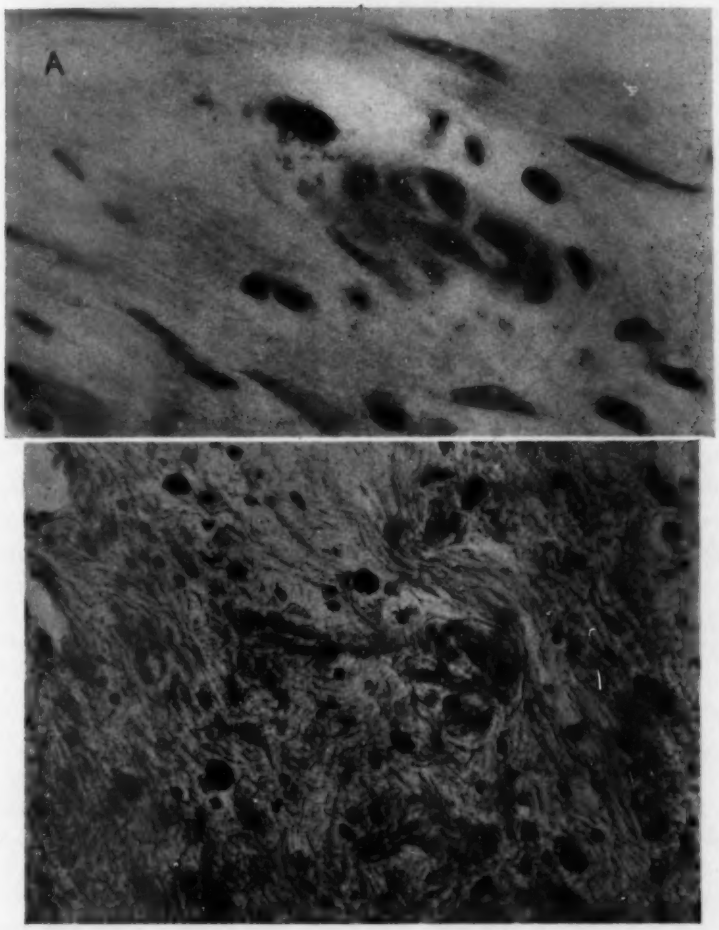

Fig. 3.—*A*, mast cell in association with a small vessel (x 1,050); *B*, mast cells in a section from lipomyxosarcoma (x 225).

certain degree are characteristic of the organ in question. This work is not yet published.

FUNCTION OF THE MAST CELL

A great variety of opinions have been offered as to the function of the mast cell. Perhaps to no other cell have such diversified functions

been ascribed. Michels[10] in his own investigations of fifteen species of lower vertebrates failed to find a specific function of the mast cell but came to two conclusions; namely, that these cells were neither phagocytic nor related to diastatic activity. Harris[13] suggested in 1900 that they produce mucin. This contention is based on the similar staining reactions of mucus and mast granules with certain aniline dyes and acidified toluidine blue. Harris, in fact, suggested that the term "mucinoblast" be used instead of "mast cell." If the Swedish investigators referred to previously are correct in their contention that toluidine blue is a specific test for heparin, then "heparinoblast" would be a more appropriate term than "mast cell."

THE MAST CELL UNDER CERTAIN PATHOLOGIC CONDITIONS

It is well known that chronic inflammation is accompanied by a marked numerical increase of mast cells. Stagnation of the lymph stream is said to favor the accumulation of these cells. In certain diseases of the skin—for example, urticaria pigmentosa[14]—there are more or less pronounced increases of these cells. This fact leads one to wonder whether or not the mast cells have any connection with histamine. It is known that heparin and histamine are liberated simultaneously in acute anaphylaxis.[15] Tissue mast cells are usually absent or extremely sparse in deeply infected regions of carcinoma but tend to accumulate in the adjoining normal tissue and in the regional lymph nodes (confirmed in this study). Michels[10] further pointed out that in tumors other than cancer there is usually an increased number of mast cells (fig. 3*B*). Bergonzini[11] noted them in tubercles of tuberculosis, and Parkhill[16] has noted their frequency in various types of granuloma. Bloom[17] has described spontaneous solitary and multiple mast cell tumors occurring in dogs. He stated that, with only four possible exceptions, no neoplasms of a similar type have been reported in the available literature on human beings and animals. The causative factor for the numerical increase of tissue mast cells under pathologic conditions is unknown.

METHOD USED IN THE PRESENT STUDY

Fresh frozen sections were made from various types of tissue which were available for study in the surgical pathologic laboratory. They were stained immediately, Terry's polychrome methylene blue stain being used.[18] With this stain, the mast granules are

13. Harris, H. F.: Philadelphia M. J. **5**:757, 1900.

14. Scolari, E. G.: Gior. ital. di dermat. e sif. **75**:1207, 1934. Sezary, A.: Lefevre, P. and Chauvillion, P.: Bull. Soc. franc. de dermat. et syph. **43**:1263, 1936. Sezary, A.: ibid. **43**:357, 1936.

15. Code, C. F.: Ann. Allergy **2**:457; 1944.

16. Parkhill, E. M.: Personal communication to the authors.

17. Bloom, F.: Arch. Path. **33**:661, 1942.

18. Terry, B. T.: J. Lab. & Clin. Med. **8**:157, 1922; Proc. Staff Meet., Mayo Clin. **1**:209, 1926.

reddish purple. The sections were then examined, a Bausch and Lomb microscope being used with a no. 10 ocular and a 21 by 8 mm. 0.50 objective. The same microscope was used throughout the study. Mast cells were counted in five representative fields in each section.

TABLE 1.—*Mast Cell Contents of Synovial Membrane (Normal)*

Age in Yr. and Sex	Source of Normal Synovial Membrane	Mast Cells in 5 High Power Fields	Comment
7 M	Left wrist	21	Carpectomy for old Volkmann's ischemic contracture
49 M	Right knee	17	Right medial meniscectomy: 7 mast cells were seen closely alined to a blood vessel
44 M	Left knee	14	Chronic poliomyelitis
56 F	Right knee	36	
53 M	Right knee	27	Hemipelvectomy for fibrosacroma of right buttock
29 F	Right knee	34	Recurrent dislocation of right patella
14 M	Right knee	36	Hemipelvectomy for osteogenic sarcoma of right femur, grade 4*
49 F	Knee	27	Right medial meniscectomy
Average		26	

*Broders' method was used.

TABLE 2.—*Mast Cell Content of Synovial Membrane (Abnormal)*

Age in Yr. and Sex	Diagnosis and Source of Abnormal Synovial Membrane	Mast Cells in 5 High Power Fields	Comment
56 M	Tuberculous tenosynovitis; wrist	64	Mast cells grouped around blood vessels in nontuberculous regions
54 M	Tuberculosis; wrist	82	In connective tissue around the lesions
15 F	Tuberculosis; left elbow	102	Not actually in the lesion, many perivascular
55 M	Tuberculosis; right knee	98	
8 M	Tuberculosis; left hip	54	
18 M	Tuberculosis; right knee	88	Not actually in the lesions
15 M	Chronic infectious arthritis with marked villous synovitis	123	Too numerous to count in certain regions; effusion of joint, 2+
20 F	Rheumatoid arthritis	46	
? F	Chronic synovitis; right astragalar scaphoid joint	61	Post-traumatic arthritis
34 M	Patellofemoral arthritis	39	Old fractured patella
Average		76	

TABLES

In the subsequent pages, tables showing the mast cell content of various tissues will be presented. The tissues entering into the formation of joints will be considered first.

Table 1 gives data for normal synovial membrane. An effort was made to take the sections from secretory regions, that is, in the neighborhood of the fat deposits. The mast cells were found in the loose

"subendothelial" layer and not among the actual lining cells of the synovial membrane.

Table 2 confirms the observations of other investigators that there is a numerical increase of tissue mast cells in tuberculosis and other chronic infections. In the English literature reviewed, we have found no reference to mast cells present in the synovial membrane. (Since this study was completed, Davies[19] has drawn attention to mast cells in the synovial membrane from the ankle joint of an ox.)

TABLE 3.—*Mast Cell Content of Fibrocartilage and Articular Cartilage*

Age in Yr. and Sex	Diagnosis and Source of Tissue	Mast Cells in 5 High Power Fields	Comment
7 M	Normal articular cartilage; carpal bones	0	Carpectomy for Volkmann's ischemic contracture
53 M	Normal medial meniscus; right knee	0	Hindquarter resection for fibrosarcoma
49 F	Torn medial meniscus	0	Degenerating
50 F	Fibrocartilage	0	Removed from hip region where vitallium cup had been in place

TABLE 4.—*Mast Cell Content of Various Tissues*

Tissue	Cases	Diagnosis	Average Number of Mast Cells in 5 High Power Fields	Comment
Appendix	15	Chronic appendicitis	28	Found in submucosa, between muscle bundles of the muscularis and in the serosa
Gallbladder	10	Chronic cholecystitis, the majority having cholelithiasis; 1 case of acute gangrenous cholecystitis	18	Chiefly confined to the serosa
Uterine cervix	9	Chronic cystic cervicitis	34	
Breast	9	Fibroadenoma; chronic cystic mastitis; chronic fibrous mastitis; adenocarcinoma	38	Fibroadenoma had highest count. No mast cells in 1 case of adenocarcinoma
Thyroid gland	6	Parenchymatous hypertrophy; adenomatous goiter; colloid goiter	0	
Kidneys	3	Hydronephrosis with chronic pyelonephritis; adenocarcinoma, grade 1*; chronic pyelonephritis	0	Parenchyma studied
Scar tissue	3	Scar	0	

*Broders' method was used.

On the basis of table 3, if mast cells are one-celled glands, it is not likely that articular cartilage and fibrocartilage provide any secretion for joints.

Data on the mast cell content of various tissues are given in table 4. None of the appendixes was removed because of symptoms related to the appendix per se but was taken in the course of operation from some other cause. It is suggested that those specimens with the higher mast

19. Davies, D. V.: Lancet **2**:815, 1946.

cell counts were examples of true chronic appendicitis if there is such a pathologic entity. From a study of sections of the gallbladder it seems that mast cells are largely confined to the serosal layer. Their number seems to vary directly as the extent of the chronic inflammation.

TABLE 5.—*Mast Cell Content of Stomach and Duodenum*

Cases	Diagnosis	Average Number of Mast Cells in 5 High Power Fields	Comment
8 (benign lesions)	Chronic peptic ulcer in 7 cases; chronic gastritis in 1	87	In connective tissue between muscle bundles adjacent to ulcer
7 (gastric cancers)	Adenocarinoma of various grades	21	Not in the lesion

TABLE 6.—*Mast Cell Content of Uterus*

Age in Yr. and Sex	Diagnosis	Mast Cells in 5 High Power Fields	Comment
46	Submucous fibromyoma	0	Not degenerating
46 F	Fibromyoma	18	Degenerating
48 F	Fibromyoma	0	Not degenerating. Five variations of stain used
34 F	Fibromyoma	0	Not degenerating
50 F	Fibromyoma	0	Not degenerating
50 F	Sections of uterine wall from preceding case	38	
58 F	Chronic metritis	35	Moderate sclerosis of vessels and musculature
32 F	Chronic metritis	31	Moderate sclerosis of vessels and musculature. Mast cells in connective tissue.
32 F	Endometriosis of myometrium	65	In connective tissue
43 F	Endometriosis of myometrium	80	
48 F	Endometriosis of surface of uterus	17	Section from uterine wall (not through the lesion)
41 F	Cystic endometrium	0	
31 F	Late proliferative phase of endometrium	0	
38 F	Late differentiated phase of endometrium	0	
54 F	Late proliferative cystic endometrium, grade 2	0	
? F	Late proliferative cystic endometrium, grade 1	0	
43 F	Early differentiated cystic endometrium, grade 2	0	
37 F	Late proliferative cystic endometrium, grade 2	0	
50 F	Late differentiated phase of endometrium	0	
66 F	Atrophic endometrium	0	
54 F	Atrophic endometrium	0	

It is noted in table 5 that mast cells are more than four times as numerous in association with benign lesions of the stomach and duodenum as with cancer of the stomach. This may be due merely to an overcrowding effect on the part of the cancer cells. One of us (J. R. M.)

has for a long time been impressed with the large number of mast cells associated with benign ulcer of the stomach.

In examination of the sections on which data are given in table 6, it was noted that mast cells were not found in uterine fibromyoma unless they were degenerating. (Bergonzini, Reich and Staemmler[20] found an abundance of mast cells in uterine fibromyoma.) They were present in the uterine wall. They were moderately increased in cases of chronic metritis and considerably increased in cases of endometriosis. Staemmler[8] found them to be extremely sparse in the uterine mucosa. In this study, no mast cells were observed in the uterine mucosa in the various phases studied. No endometrial tissue studied had been taken at the time of actual menstruation.

TABLE 7.—*Mast Cell Content of Prostate*

Cases	Diagnosis	Average Number of Mast Cells in 5 High Power Fields
7 (Benign lesions)	Adenomatous hyperplasia	24
2 (Cancers)	Adenocarcinoma, grade 3*	0

*Broders' method was used.

TABLE 8.—*Mast Cell Content of Lymph Nodes (Cases of Carcinoma); Cases of Adenocarcinoma of Breast*

Cases	Diagnosis	Average Number of Mast Cells in 5 High Power Fields
5	No nodal involvement	110
2	Nodal involvement	4

TABLE 9.—*Mast Cell Content of Head and Neck*

Cases	Diagnosis	Average Number of Mast Cells in 5 High Power Fields	Comment
12 (Benign lesions)	Varied	58	In connective tissue. Many lining blood vessels
8 (Cancers)	Varied	64	Not usually in the lesion proper

More sections of cancer of the prostate (table 7) would have to be studied before any opinion could be given regarding whether or not more mast cells are present in noncancerous than in cancerous lesions of the prostate.

From a study of the sections on which data are given in table 8 it appears that a large number of mast cells are found in lymph nodes in the neighborhood of carcinoma when the node is uninvolved, but that they are reduced in number or absent when the node is involved.

20. Bergonzini, Reich and Staemmler; cited by Michels,[10] p. 345.

There is a numerical increase of mast cells in association with lesions of the head and neck, both cancerous and noncancerous (table 9). In the cancerous lesions, the mast cells are not usually in the lesion proper but in the neighboring tissues.

As far as thyroid (human) tissue is concerned (table 4), this study confirms the work of Ekman and Naumann,[21] who found no mast cells in normal thyroid glands or in those involved in exophthalmic goiter. The results for the breast and for scar tissue (table 4) are in accord with those of other workers.

TABLE 10.—*Mast Cell Content of Miscellaneous Tissues*

Age in Yr. and Sex	Diagnosis and Source of Tissue	Mast Cells in 5 High Power Fields	Comment
64 M	Papillary squamous cell carcinoma, grade 2*; ureter	118	Not in the lesion
52 F	Chronic pyelonephritis; nephrolithiasis	70	Mast cells in section of ureter 5 cm. from pelvis
30 F	Regional ileitis	103	Between muscle bundles along blood vessels
44 M	Tuberculous kidney; section of pelvis	71	
34 M	Tuberculosis; right and left epididymis	112	
48 M	Periarteritis nodosa	18	Biopsy, left calf. Mast cells in connective tissue
51 M	Right inguinal hernia	4	Hernial sac
53 M	Left inguinal hernia	0	Hernial sac
68 M	Left inguinal hernia	3	Hernial sac
65 M	Adenocarcinoma, grade 1*; sigmoid	47	Between muscles, along blood vessels
? M	Adenocarcinoma, grade 1*; rectum	47	In connective tissue of muscle coats
29 F	Fallopian tube, normal	22	Patient diabetic
29 F	Umbilical cord	0	Same case as preceding
29 F	Placenta	0	Same case as preceding
? M	Mediastinal cyst (simple)	6	In connective tissue
60 M	Lipomyxosarcoma, grade 1*	50	In stroma, not in tumor proper
76 M	Lipoma pectoral region	11	In stroma

*Broders' method was used.

Data concerning miscellaneous tissues are given in table 10.

Sections from four lungs removed for varying pathologic conditions were studied for their mast cell content. No definite counts could be made because of the difficulty of differentiating the mast cells from the large macrophages.

CHEMICAL EXPERIMENT

Seventy-five cubic centimeters of fluid blood were aspirated from a knee four days after medial meniscectomy. Two hours and thirty-five

21. Ekman, C. A., and Naumann, B.: Acta path. et microbiol. Scandinav. **22**:271, 1945.

minutes later, this hemarthrosis was still fluid. Ten cubic centimeters of a 1 per cent solution of protamine was added to 10 cc. of the blood. A definite coagulum, both microscopic and macroscopic, appeared. It is known that protamine annuls the action of heparin, both in vitro and in vivo.[22] This suggests that heparin is at least partly responsible for the prevention of clotting in hemarthrosis.

SUMMARY

One hundred and seventy fresh frozen sections of various human tissues were stained with Terry's polychrome methylene blue stain and examined for their mast cell content.

Histogenous mast cells were found in connective tissue wherever it was studied.

Mast cells were found in normal synovial membrane, in the "subendothelial layer," averaging 26 per 5 high power fields. Their number was considerably increased in this location in cases of chronic inflammation and tuberculosis, averaging 76 per 5 high power fields.

Mast cells were not found in tissues which were the site of acute inflammation. They were not found in fibrocartilage, articular cartilage, scar tissue, endometrium, umbilical cord, placental tissue, thyroid tissue or renal parenchyma.

Mast cells were found in great numbers in lymph nodes uninvolved by carcinoma.

Mast cells were found to be more than four times as numerous in association with noncancerous lesions of the stomach and duodenum as with cancer of the stomach.

It is suggested that mast cells of the synovial membrane, through their secretion of heparin, may play a part in keeping hemarthrosis fluid. A biochemical study would be necessary to test this point. However, from indirect evidence it would seem likely that heparin from the mast cells is the anticoagulant in synovial fluid, since Jorpes[23] and Bergstrom[24] have demonstrated that the chondroitic and mucoitin monosulfuric acids present in cartilage and mucin have no anticoagulant activity.

---

22. Chargaff, E., and Olson, K. B.: J. Biol. Chem. **122**:153, 1937.
23. Jorpes, E.: Naturwissenschaften. **23**:196, 1935; Biochem. J. **29**:1817, 1935.
24. Jorpes, E., and Bergstrom, S.: J. Biol. Chem. **118**:447, 937; footnote 7.

PANCREATITIS ACCOMPANYING HEPATIC DISEASE IN DOGS FED A HIGH FAT, LOW PROTEIN DIET

STUART LINDSAY, M.D.
SAN FRANCISCO
C. ENTENMAN, PH.D.
AND
I. L. CHAIKOFF, M.D.
BERKELEY, CALIF.

INASMUCH as the cause of pancreatitis occurring in man is often obscure, it is of value to record here the finding of pancreatitis in dogs fed high fat, low protein diets. Dogs were fed daily 7 Gm. of lean meat and 10 Gm. of lard per kilogram of body weight. In addition each dog received 50 Gm. of sucrose, 5 cc. of "galen B,"[1] 3 cc. of "sardilene",[2] 14 Gm. of bone ash, 2 Gm. of Cowgill's[3] salt mixture and 20 Gm. of "cellu flour" per day. Fatty livers have been observed as early as thirty-five days in dogs fed this diet. In the 13 dogs recorded in the table, the fatty acid content of the livers varied from 12 to 37 per cent. Grossly visible pancreatic lesions were observed in 11 of the dogs. In these lesions the interstitial tissue appeared to be primarily involved, with the acinous tissue only secondarily affected. Two phases of the process are described in the following pages.

ACUTE PANCREATITIS

In the acute stage (fig. 1) the pancreas was congested, edematous and firm. Scattered throughout the peripancreatic fat and interlobular septums were numerous tiny foci of opaque yellow fat necrosis. Thrombosis of large vessels was grossly visible. Microscopic examination revealed considerable variation in the severity of the process. In some dogs only small foci of acute interstitial and acinous necrosis were found; a few neutophilic leukocytes surrounded each necrotic area. In others the interstitial and peripancreatic fat necrosis was extensive, and in these areas an abundant fibrinopurulent exudate was found. Acinous

---

From the Divisions of Pathology (San Francisco) and Physiology (Berkeley) of the University of California Medical School.

Aided by a grant from the Sugar Research Foundation.

1. (Montgomery, M. L.; Entenman, C.; Chaikoff, I. L., and Nelson, C.: J. Biol. Chem. **137**:693, 1941).

2. Each cubic centimeter of "sardilene" contained not less than 100 A.O.A.C. chick units of vitamin D and 600 U.S.P. units of vitamin A.

3. Cogwill, G. R.: J. Biol. Chem. **56**:725, 1923.

necrosis was observed only in the periphery of the lobules. There were no alterations of the islets or ducts. The blood vessels were normal ex-

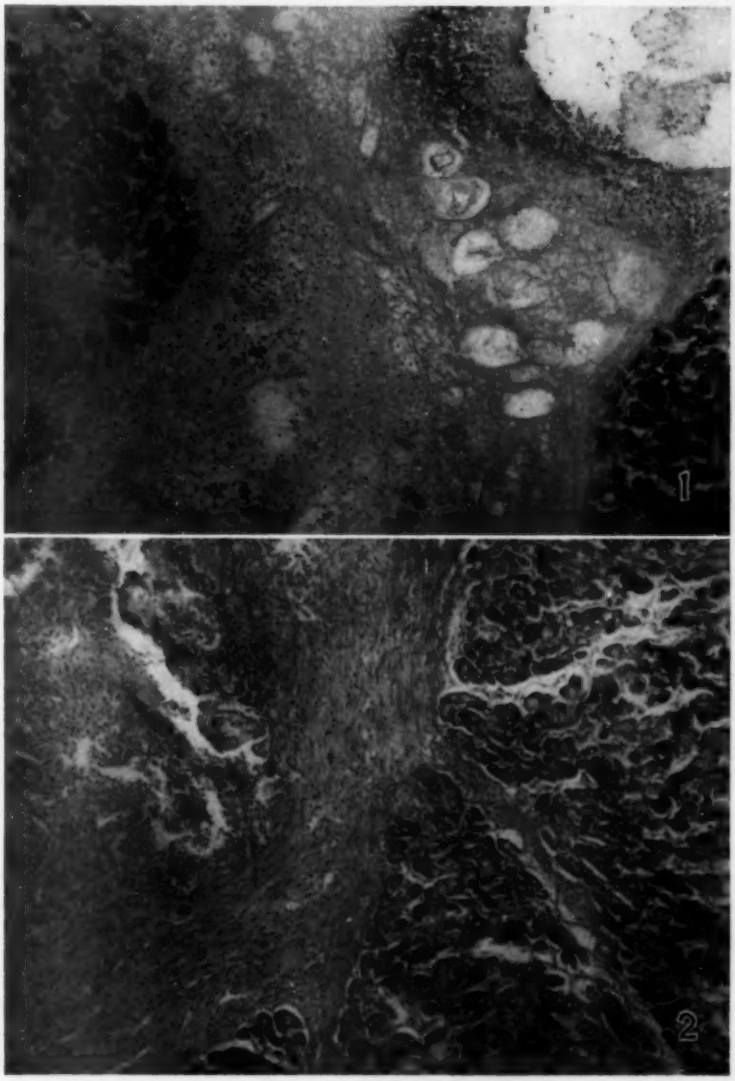

Fig. 1—Acute interstitial pancreatitis with fat necrosis. Hematoxylin and eosin stain; x 79.

Fig. 2.—Subacute pancreatitis with interlobular scarring. Hematoxylin and eosin stain; x 79.

cept where their walls were secondarily affected by the acute inflammatory reaction about them.

SUBACUTE PANCREATITIS

This type of lesion was characterized grossly by extensive translucent fibrous scarring of the interlobular and peripancreatic tissues, which accounted for the nodular surfaces of the glands. On microscopic examination (fig. 2) this fibrous material was found to be of recent origin and heavily infiltrated with lymphocytes. In addition there were foci of fat necrosis with a neutrophilic leukocyte infiltration similar to that observed in the acute stage. Numerous macrophages containing lipid material were present in these areas of necrosis. There was no squamous metaplasia of the ductal epithelium.

The Effect of a High-Fat, Low-Protein Diet on the Liver and the Pancreas

	Body Weight, Kg.			Liver			Pancreas	
Dog	Weeks on Diet	Initial	Final	Wet Weight, Gm.	Total Fatty Acids, Per Cent of Wet Weight	Microscopic Observations*	Gross Finding	Microscopic Observation
18	23	9.2	8.5	580	31.7		Pancreatitis	
24	23	9.0	7.8	335	16.6		Pancreatitis	
30	31	6.7	8.2	365	20.2	Intralobular and periportal fibrosis	Pancreatitis	Acute pancreatitis
36	25	14.2	16.2	456	37.2		Pancreatitis	
38	21	8.9	13.0	478	17.2		Normal	
40	33	8.9	12.7	535	26.6		Pancreatitis	
42	27	9.0	10.7	405	17.4	No fibrosis	Pancreatitis	Subacute pancreatitis
46	34	12.9	14.3	720	18.5	No fibrosis	Pancreatitis	Acute pancreatitis
48	29	9.0	12.7	621	15.3		Pancreatitis	
50	30	9.2	12.5	575	22.6	No fibrosis	Normal	Normal
52	14	10.0	11.4	652	27.2	Early fibrosis	Pancreatitis	Acute pancreatitis
54	38	6.0	4.5	160	12.0	Intralobular and periportal fibrosis	Pancreatitis	Subacute pancreatitis
56	17	16.8	15.5	910	21.7		Pancreatitis	

*Absence of an observation implies that no microscopic studies were made.

HEPATIC LESION

Extensive fat infiltration was found in the livers of all dogs (table). In addition, periportal and/or diffuse intralobular fibrosis was observed in several of the livers examined. These changes resembled closely those described earlier in dogs fed high fat, low protein diets.[4]

COMMENT

The atrophic pancreatitis and fatty liver occurring in rats fed a low protein, high fat diet has been reported by Grossman, Greengard and Ivy.[5] Chronic cystic pancreatitis and fatty liver have also been observed by Gillman and Gillman[6] in rats fed mealie pap and sour milk; but these pancreatic lesions, which appeared some time after the

4. Chaikoff, I. L.; Eichorn, K. B.; Connor, C. L., and Entenman, C.: Am. J. Path. **19**:9, 1943.

5. Grossman, M. I.; Greengard, H., and Ivy, A. C.: Am. J. Physiol. **138**:676, 1943.

6. Gillman, T., and Gillman, J.: Arch. Int. Med. **76**:63, 1945.

development of fatty liver in the rat, bore no resemblance to those described here for the dog. The Gillmans found that in their rats the primary involvement was in the glandular alveoli and intralobular connective tissue, whereas the pancreatitis observed here in the dog is characterized by a primary reaction in the interlobular connective tissue and adjacent margins of lobules. In these dogs the parenchymatous tissue was relatively undamaged.

The pancreatitis recorded here for the dog resembles, at least pathologically, that encountered in man suffering from alcoholism. Clark found pancreatic disease in 27 of approximately 150 consecutive cases of alcoholism seen at necropsy.[7] The pancreatic lesions varied from minimal fat necrosis to extensive necrosis with acute inflammation and hemorrhage. Pancreatic fibrosis of varying degree was observed in 24 of the 36 cases examined microscopically. In the majority of Clark's cases the liver was enlarged and fatty, while hepatic cirrhosis was present in 17. Among the 356 fatal cases of hepatic cirrhosis in persons with alcoholism studied by Kirshbaum and Shure,[8] well marked pancreatic fibrosis was encountered in 129, or 36 per cent.

As can be seen from the table, all the dogs had very fatty livers (15 per cent fatty acids or more), and fibrosis was detected in 3 of the 6 livers examined microscopically. It is possible that fibrosis or cirrhosis would have been seen more frequently had we done microscopic studies in all cases.

Although the Gillmans[6] were able to state with certainty that the hepatic disease preceded the pancreatic lesions in their rats, no such correlation could be made either in our dogs or in the clinical material cited. This relation between pancreatic and hepatic disease merits careful study, since it has already been demonstrated that certain pancreatic extracts can profoundly modify the reactions of the liver.[9] Further studies designed to elucidate this hepatopancreatic relation are now in progress in this laboratory.

SUMMARY

Pancreatitis occurring in dogs fed a high fat, low protein diet is described. It was interstitial in type and consisted of acute necrosis with secondary inflammation and scarring. Acinous involvement was secondary and minimal. The pancreatic lesion was associated with heavy fat infiltration and early fibrosis of the liver.

---

7. Clark, E.: Am. J. Digest. Dis. **9**:428, 1942.
8. Kirshbaum, J. D., and Shure, N.: J. Lab. & Clin. Med. **28**:721, 1943.
9. Entenman, C.; Chaikoff, I. L., and Montgomery, M. L.: J. Biol. Chem. **155**:573, 1944.

CHOLECYSTITIS

A Study of Intramural Deposits of Lipids in Twenty-Three Selected Cases

RODGER E. WEISMANN, M.D.
Fellow in Surgery, Mayo Foundation
AND
JOHN R. McDONALD, M.D.
ROCHESTER, MINN.

ALTHOUGH less frequent in occurrence than cholesterosis, or "strawberry gallbladder," large intracellular deposits of lipoid materials are not uncommonly observed in the subserosal layers of diseased gallbladders. The significance of these intramural deposits is not generally known. It is thought that further study of the phenomena might serve to identify unknown incitements in the genesis of cholecystitis. In addition, the data obtained would be considered useful in clarifying the problem of the proper surgical management of advanced lesions of the gallbladder.

The present study was concerned mainly with the pathologic picture in 23 cases of this variant of cholecystitis, with special reference to the distribution, the amount and the type of lipids encountered. Chemical determinations of the lipid content of the tissue were employed to supplement the microscopic investigations.

GENERAL CONSIDERATIONS

Pathogenesis.—The cause of cholecystitis, with or without calculi, is not generally agreed on. Irritants capable of producing inflammatory changes can be divided into two groups: (1) infectious and (2) noninfectious (inorganic or organic chemicals or metabolites). Obstruction of the cystic duct or of the distal part of the biliary tract obviously is of major importance in the production of alterations of the physiology of the system.[1] Certain evidence exists that vascular disturbances are of prime importance in some instances.[2]

The theory that inflammatory disease of the gallbladder is primarily a bacterial infection, with superimposed alterations of that organ and

---

From the Section on Surgical Pathology, Mayo Clinic.

1. *(a)* Bisgard, J. D., and Baker, C. P.: Tr. Am. S. A. **58**:572, 1940; Ann. Surg. **112**:1006, 1940. *(b)* Womack, N. A.: J. Kansas M. Soc. **42**:501, 1941.

2. *(a)* Andrews, E.: Arch. Surg. **31**:767, 1935. *(b)* Denton, J.: ibid. **14**:1, 1927. *(c)* Feinblatt, H. M.: New England J. Med. **199**:1073, 1928.

secondary disorders characterized by the formation of calculi, has become deeply ingrained in all medical literature and textbooks and has apparently remained the most popular concept of the pathogenesis to date.

In the past thirty years, however, there have been two important developments in the study of the genesis of cholecystitis which have renewed interest in this subject. First, there was the discovery of the nature of cholesterosis or subepithelial intracellular and extracellular depositions of cholesterol or, occasionally, of other unidentified lipids.[3] Second, most investigators have been unable to recover pathogenic micro-organisms from either the contents or the substance of the majority of diseased gallbladders.[4]

While the view that infection is the sole cause of cholecystitis still prevails and largely governs the therapeutic principles, the idea that the process is primarily a chemical one is gaining increased support.[5] Clinical and experimental observations assigning a minor or insignificant role to primary infection of the gallbladder have been published by numerous investigators.[6] Aronsohn and Andrews[6a] were unable to obtain any consistent results in attempts to induce pathologic changes in the wall with bacterial irritants. Bacteria, even when present in bile in overwhelming numbers, failed to produce any significant inflammatory reaction unless they were of unusual virulence. Aronsohn and Andrews expressed a belief that the human gallbladder in almost all cases shows no signs of infection and that no micro-organism can be found in specially prepared and stained preparations. Furthermore, sepsis is rarely, if ever, a complication of cholecystitis. The experiences of Womack and Bricker[6b] led them to consider that while bacterial invasion of the gallbladder is possible, it appears to be a complementary or a secondary process in most cases and represents a complication of the primary disorder.

It is a frequent clinical observation that there is often little or no evidence of an infectious or a septic process in patients with severely diseased gallbladders which are surrounded by abscesses; the surgeon

---

3. Boyd, W.: Brit. J. Surg. **10**:337, 1923. Elman. R., and Graham. E. A.: Arch. Surg. **24**:14, 1932. Illingworth, C. F. W.: Brit. J. Surg. **17**:203, 1929. MacCarty, W. C.: Ann. Surg **51**: 651, 1910. Mentzer, S. H.: Am. J. Path. **1**:383, 1925.

4. Andrews, E., and Henry, L. D.: Arch. Int. Med. **56**:1171, 1935. Drennan, J. G.: Ann. Surg. **76**:482, 1922. Osler, W., and McCrae, T.: Modern Medicine: Its Theory and Practice, ed. 2, Philadelphia, Lea & Febiger, 1914, vol. 3, pp. 550-551. Andrews.[2a] Feinblatt.[2c]

5. Gatch, W. D.; Battersby, J. S., and Wakim, K. G.: J. A. M. A. **132**:119, 1946.

6. *(a)* Aronsohn, H. G., and Andrews, E.: Surg., Gynec. & Obst. **66**:748. 1938. *(b)* Womack, N. A., and Bricker, E. M.: Arch. Surg. **44**:658, 1942. *(c)* Denton.[2b] *(d)* Feinblatt.[2c]

may be surprised to break into such an abscess during the routine removal of a diseased gallbladder. Touroff[7] analyzed a series of gallbladders which were the sites of complicated acute or subacute disease and noted the striking lack of correlation between clinical manifestations and pathologic changes. Cultures of the contents of the abscesses frequently show no pathogenic organisms. Wound infections following surgical attacks are unusual. Pyelophlebitis, secondary hepatic abscesses or general sepsis from such localized processes are rare complications, in contradistinction to perforating processes of the appendix. The destruction may go on to the formation of fistula before its discovery is possible clinically.

Necrotizing or gangrenous inflammatory lesions are reported by all authors but apparently are of infrequent occurrence.[8] A patchiness of distribution, with marked cyanotic changes in the more severe pathologic process, seems to characterize this unusual lesion. For this reason, a primary vascular disturbance has been suggested as an etiologic factor. Womack and Bricker[6b] pointed out that gangrene of the gallbladder occurs in the presence of abundant collateral circulation with the liver and cannot be induced by ligation of the cystic artery or the cystic vein. He expressed a belief that the necrosis is the result of direct injury of tissue and designated it as a phenomenon of necrobiosis.

Numerous experimental studies have shown that lesions identical with those of cholecystitis of human beings, both that occurring in the presence of calculi and that independent of stones, can be produced with exogenous and endogenous noninfectious irritants of a chemical nature.[9] These may be products of body metabolism; they will cause severe inflammatory changes when applied directly or indirectly to the gallbladder wall. Many of the phenomena observed in the operating room or the pathologic laboratory have been reproduced in this experimental work. The primary importance of the irritating effect of concentrated or imprisoned bile or its constitutents is clearly apparent. When pancreatic juices are added, extensive pathologic changes result.[10]

Pathologic Features.—In both the acute and the chronic form of cholecystitis the inflammatory changes of the gallbladder are strikingly

---

7. Touroff, A. S. W.: Ann. Surg. **99**:900, 1934.

8. *(a)* Baumgartner, C. J.: Surg., Gynec., Obst. **49**:780. 1929 *(b)* Cowley, L. L.: and Harkins, H. N.: ibid. **77**:661, 1943. *(c)* Glenn, F., and Moore, S. W.: Arch. Surg. **44**:677, 1942.

9. *(a)* Mann, F. C.: Ann. Surg. **73**:54, 1921. *(b)* Wolfer. J. A.: Surg., Gynec. & Obst. **53**:433, 1931. *(c)* Womack, N. A., and Bricker, E. M.: Proc. Soc. Exper. Biol. & Med. **45**:710, 1940. *(d)* Bisgard and Baker.[1a] *(e)* Gatch and others.[8] *(f)* Aronsohn and Andrews.[9a] *(g)* Womack and Bricker.[9b] *(h)* Mann.[9a] *(i)* Wolfer.[9b] *(j)* Womack and Bricker.[9c]

10. Bisgard and Baker.[1a] Wolfer. [9b]

dissimilar to inflammatory processes induced by bacteria or bacterial irritants in other organs.[11] Acute cholecystitis presents a microscopic picture characterized by marked inflammatory edema, with capillary engorgement, and relative paucity of acute inflammatory cells—that is, polymorphonuclear leukocytes. The mucosa, except for edema, shows little destruction, and the most marked findings are present in the subserosal portion of the wall. The thickening of the wall is largely due to edema and vascular engorgement. Rarely, if ever, can bacteria be identified in the microscopic sections made of these specimens. Ulceration of the mucosa is rare, and when present, is due to necrosis caused by pressure of tightly packed calculi.[2a] "Empyema" of the gallbladder does not occur, according to several authors,[2] and the so-called purulent exudate reported in the lumen of diseased gallbladders by so many German observers is in reality a cloudy emulsion containing high concentrations of cholesterol, other lipids, calcium carbonate and mucoid debris. Few, if any, pus cells can be identified in smears of this liquefied debris. Cultures of contents are sterile in 60 per cent or more of the cases.

Much has been written concerning the importance of the so-called Rokitansky-Aschoff sinuses which are so frequently seen in the diseased gallbladder. Recently the concept that these structures are in reality diverticula[12] seems to indicate a more significant role than heretofore has been ascribed to them. Since an increase in the number and the size of these intramural structures is seen in chronic inflammatory disease, it appears that they may, in some manner, play a role in the genesis of lesions of the gallbladder. These structures may form channels by which bile can escape from the lumen of the gallbladder into external coats of the organ, or they may become niduses for "abscesses," calculi or collections of bile pigment, which are frequently observed in advanced pathologic processes. Bile imprisoned in these crypts would become exceedingly toxic. It has been suggested that perforations develop as a result of destructive processes in these crypts or diverticula.[13]

Bile.—The organic constitutents of bile are of primary importance in the consideration of possible sources of chemical irritants of the gallbladder wall. Of these constitutents, bile salts and cholesterol seem to be the most potent, while bile pigments and lecithin probably have little toxicity.[14] Bile salts reach a concentration of 7 to 10 per cent in gallbladder bile.[6a]

---

11. Boyd, W.: Surgical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1942.

12. Robertson, H. E., and Ferguson, W. J.: Arch. Path. **40**:312, 1945.

13. Glenn and Moore.[9c] Robertson and Ferguson.[12]

14. Aronsohn and Andrews.[9a] Womack and Bricker.[9c]

Cholesterol, which occurs in bile normally only in the free state, is the most toxic lipid present in bile.[9c] Lecithin is constantly present in slightly less amounts, as are fatty acids and probably some neutral fat. Cholesterol, however, occurs in hepatic bile in concentrations of 20 to 200 mg. per hundred cubic centimeters and at levels usually lower than those in plasma. Concentration of the gallbladder bile results in increases of the cholesterol fraction ranging from two to fifteen times.

Changes Due to Lipid Irritants.—Recently it has been shown that certain lesions of an inflammatory nature involving various organs or tissues are associated with either a local or a systemic disturbance of lipid metabolism. While a cause and effect relationship, in most instances, still remains to be shown, it appears that if the lipid or its breakdown products are not the primary irritant, they contribute to the intensity, duration and development of a certain characteristic histopathologic picture.

In general, certain histologic alterations have been noted in association with lesions thought to be incited by lipid irritants.[15] Lymphocyte and plasma cell infiltrations are characteristic of many inflammatory processes in which lipids have been the inciting factor or have been intimately associated with the process. The role of these cells cannot be identified with any degree of certainty, but the observations that they are commonly present in many inflammatory processes has led many pathologists to suspect a common stimulant or reactive phenomenon. While fibroblastic reaction is universally present as a result of an inflammatory process of any type, the activity is characteristically excessive and, in many cases, extreme in amount. The proliferative reaction may develop slowly and persist indefinitely. Thus it is strikingly similar to foreign body tissue reaction, and the two may represent the same process. The presence of excessive amounts of cholesterol in tissues characteristically causes a fibroblastic reaction.[16]

Specifically, three histopathologic examples of lesions due or related to irritants of lipoid materials are commonly recognized. The first and commonest, that of fat necrosis, occurs in adipose tissue in almost any location. The causes may be several, but the presence of a hydrolytic enzyme in areas of fat necrosis has been established by experimental study. The lipase may be released in the breakdown of nearby cells, or it may be present as a result of escape of activated digestive juices, that is, those which contain pancreatic enzymes.[17] Bile itself can either provide a hydrolyzing effect or activate tissue lipase so as to produce

15. *(a)* Hirsch, E. F.: Arch. Path. **31**:516, 1941. *(b)* Hirsch, E. F., and Weinhouse, S.: ibid. **30**:1079, 1940.

16. Kimmelstiel, P., and Laas, E., cited by Hirsch,[15a] p. 523.

17. Rewbridge, A. G.: Arch. Path. **12**:70, 1931.

hydrolysis or split of the fatty acid esters in adipose tissue.[18] In this lesion the irritating effect of the elaborated free fatty acids gives rise to the inflammatory process and results in the characteristic pathologic picture. In a review Hirsch pointed out that the effect of fatty acids on tissues depends on (1) the degree of acidity produced, (2) the kind of soap formed and (3) the chemical structure of the fatty acid. The degree of necrosis may be indirectly proportional to the hydrogen ion concentration of the resulting solution of free fatty acids. There is marked difference in the intensity and duration of the inflammatory picture which results from the effects of the various fatty acid radicals. It is apparent, therefore, that fat necrosis represents a purely chemical inflammation, and it is universally recognized as such.

Second, other more unusual examples are encountered with the so-called lipoid granulomas. In recent years many pathologists have recognized that certain granulomatous lesions observed in the lung, the prostate gland, the breast, the thyroid glands and the fallopian tubes could not be ascribed to a specific infectious agent. Repeated studies on many of these pathologic processes have given many clues which suggest a chemical irritant as the inciting agent. The lesions of "lipoid" pneumonia have now been universally recognized as a result of the local direct effect of exogenous fats and oils which reach the lung by the bronchial route. A characteristic granulomatous process of varying intensity and duration results. Characteristically, there are present large numbers of histiocytes and other inflammatory cellular elements containing large amounts of engulfed fatlike substance. Here, again, it is probably the fatty acid fraction which produces the irritating effect and which sets up the chain of events similar to or identical with that seen in fat necrosis. In the case of unhydrolyzed fats or oils, such as liquid petrolatum, the reaction is less intense, and it simulates more closely the foreign body granulomatous process. Giant cells are common in lesions associated with either etiologic factor.

Comedomastitis, a variant of chronic cystic mastitis, in which an intense fibroblastic exudative and giant cell reaction is noted, has been thought to be the result of tissue reaction to elements of retained or imprisoned secretion of the milk-producing acini. This secretion is known to be high in lipids, and these substances may be responsible for the characteristic picture. In cases of the unusual condition known as plasma cell mastitis, both experimental and clinical data point strongly to the irritating effect of retained secretion. Conclusive proof was not available at the time that Cromar[19] studied this problem in 1940. The

18. Schweizer. R.: Schweiz. med. Wchnschr. **54**:265, 1924.

19. Cromar, C. D. L.: Plasma Cell Mastitis: A Clinical and Pathological Consideration of Twenty-Four Cases, Thesis. University of Minnesota Graduate School, 1940.

striking similarity to granulomatous lesions of the thyroid gland, which Ewing chose to designate as benign granuloma of the thyroid gland, has impressed many investigators. The histologic similarity would suggest a chemical etiologic factor for the latter type of lesion, perhaps closely allied to the irritant involved in the mammary lesions.

A study of the chemical and pathologic aspects of granulomatous lesions of the prostate gland has been published.[20] There was no evidence of tuberculosis or other specific lesions in the cases reported, and it was concluded that "granulomatous prostatitis" resulted from the mixed toxic effects of obstructed prostatic secretion and resident microorganisms. It was pointed out that prostatic secretion contains large amounts of sphingomyelin, in addition to proteins and nucleic acids. A histopathologic similarity to plasma cell mastitis was noted, suggesting further an allied type of inflammatory process.

Lastly, tumefactive lesions known as xanthomas are seen in connective tissues in almost any location in the body and may be unassociated with any demonstrable systemic disturbance of lipid metabolism. The gross and the microscopic pictures are quite characteristic, and the excessive amounts of intracellular lipids, the varying degrees of fibrosis and the presence of giant cells help immediately to classify such lesions with other pathologic processes ascribed to lipid disturbances. The lipid which is present stains as cholesterol ester, and repeated chemical analysis of the tissue shows an elevated concentration of cholesterol and its esters. The presence of peculiar giant cells with nuclei arranged in peripheral fashion, the so-called Touton cells, which are not seen in any other location, has been noted. A xanthomatous tumor may be more of a granuloma than a neoplasm.[11]

METHODS AND MATERIALS

This study consisted of a review of the available clinical data and an exhaustive pathologic examination of the tissue removed at operation in 23 cases of inflammatory disease of the gallbladder. The selection of the cases was made on the basis of histopathologic evidence of excessive intramural intracellular deposits of lipoid material. Cases in which only cholesterosis was evident were not included.

No attempt was made to determine the incidence of inflammatory lesions of the gallbladder showing evidence of excessive deposits of lipoid material. In most instances the factors of obstruction of the cystic duct and vascular disturbances could not be evaluated. Bacteriologic studies were not possible or available except in 2 instances.

To corroborate further our histologic observations it was helpful to carry out quantitative chemical determinations of lipids present in the wall of the gallbladder in 5 specimens. For this additional study, cases called typical from a histopathologic standpoint were selected. In addition, in 7 control cases tissue was analyzed chemically for amounts and types of lipids present. The control group included 2 cases of extremely minimal chronic cholecystitis without cholesterosis, 4 cases of very mild chronic

20. Tanner, F. H., and McDonald, J. R.: Arch. Path. **36**:358, 1943.

cholecystitis with some degree of cholesterosis and 1 case of subacute purulent cholecystitis with necrosis. This part of the study was to provide control data which could not be found in the literature.

To obtain the clinical data utilized in this study, a review was made of the clinical records, the operative reports and the laboratory data.

The pathologic investigation was made on the specimen removed at operation in each case. The histologic studies were made from a number of blocks in each case. Blocks were taken from areas in the wall of the gallbladder where gross evidence of the presence of excessive lipoid material was noted. This evidence consisted of localized areas, on cut section, of yellow, yellowish brown or yellowish green discoloration and of parts which contained "abscesses" or marked softening in the subserosal or muscular layers. From portions of each block, fixed paraffin sections stained with hematoxylin and eosin were made for routine study. From the remaining portion of each block a frozen section was made and stained with sudan IV (scarlet red), the use of fat solvents being avoided in the processing of these sections. In a few instances frozen sections stained with nile blue were made, but the latter did not contribute to the study. In 4 instances it was possible, in addition, to study the fresh tissue with a fresh frozen section, polychrome methylene blue stain being utilized.

Use of the histologic technic was made principally in order to investigate the tissue reaction, the cellular exudates and the location of the lesions and to identify certain anatomic disturbances or peculiarities which could be associated with the genesis of lesions of the wall of the gallbladder in which lipids were excessive and gave the appearance of having participated in the end process. Excessive lipid content when present could usually be suspected on examination of the hematoxylin and eosin section, so that the special sections, as a rule, contributed only confirmatory evidence. Positive identification of the actual lipids observed in the microscopic studies could not be accurately made with available staining methods.

The chemical studies provided the most valuable method of identifying the fatty substances present in large amounts in these specimens. The method used in these analyses was improvised from suggestions received from Dr. M. H. Power,[21] of the section on biochemistry of the Mayo Clinic. Extraction of the tissue lipids was made with 50 cc. of a 3:1 mixture of alcohol and ether. One gram of tissue was prepared in the solvent by thorough grinding by hand with fine sand and mortar and pestle. After washing and filtration, a portion of the solution was analyzed after the method of Bloor[22] for fractional determination of plasma lipid. Another portion was utilized for determining lecithin content after the method of Youngburg and Youngburg.[23]

The quantitative determination of the lipids has been expressed as grams of the reported fraction in 100 Gm. of tissue. The fatty acid fraction represented the sum total of all available fatty acid radicals in the solution, including those occurring as neutral fat, as esters of cholesterol or in the phospholipid conjugations. The data for cholesterol fractions were the total cholesterol available and that fraction of the total cholesterol present in ester form, indicated by the volume of the cholesterol esters. The value for total lipids present was determined by obtaining the sum of the cholesterol and the fatty acid fractions.

---

21. Power, M. H.: Personal communication to the authors.

22. Bloor, W. R.: J. Biol. Chem. **77**:53, 1928. Bloor, W. R.; Pelkan, K. F., and Allen, D. M.: ibid. **52**:191, 1922.

23. Youngburg, G. E., and Youngburg, M. V.: J. Lab. & Clin. Med. **16**:158, 1930.

RESULTS

Clinical Data.—The pertinent clinical data in the 23 cases in which there occurred advanced inflammatory changes, with histologic evidence that the lipid content of the wall was excessive, are summarized in tables 1, 2, 3 and 4.

In the large majority of cases, the symptoms, the physical signs or the laboratory data did not indicate the severity of the actual inflammatory involvement of the gallbladder (table 1). In 1 case the abdomen was explored for carcinoma of the stomach and an unsuspected severely inflamed gallbladder was encountered in addition to the neoplastic gastric lesion.

TABLE 1.—*Significant Clinical and Laboratory Data in the 23 Cases Studied*

Symptom or Finding	Cases
Pain in upper part of abdomen	23
Abdominal tenderness	18
Digestive disturbances	22
Mass in right upper quadrant	10
Jaundice	2
Nonfunctioning gallbladder (cholecystogram)	11
Poorly functioning gallbladder (cholecystogram)	1
Leukocyte count less than 10,000	11
Leukocyte count 10,000 to 12,000	4
Leukocyte count 12,000 to 15,000	5
Leukocyte count more than 15,000	3
Additional conditions present	
Carcinoma of stomach	1
Gout	1

TABLE 2.—*Surgeon's Findings in the 23 Cases Studied*

Finding*	Cases
"Empyema" or obstruction of cystic duct	7
Pericholecystic abscess without definite evidence of rupture	4
Rupture of gallbladder with impending fistula	16
No pericholecystitis or evidence of complications	3
Dilation of common duct, with choledocholithiasis	2

*One or more of these findings were observed in each case.

TABLE 3.—*Surgical Procedures in the 23 Cases Studied*

Operation	Cases
Cholecystectomy	17
Cholecystostomy	1
Primary cholecystostomy, with cholecystectomy done later	2
Cholecystectomy and choledochostomy	3
Total	23

TABLE 4.—*Surgical Pathologic Reports in the 23 Cases Studied*

Pathologic Report	Cases
Cholelithiasis	23
Acute gangrenous cholecystitis	4
Acute purulent cholecystitis	3
Subacute purulent cholecystitis	8
Subacute catarrhal cholecystitis	4
Chronic purulent cholecystitis	2
Chronic catarrhal cholecystitis	2
Total	23

Table 2 summarizes the descriptions of the pathologic processes encountered, given by the operating surgeon. The surgeon was able to perform cholecystectomy in all but 3 cases, even though advanced complicated lesions of the gallbladder were present (table 3). There was one death in the hospital, in this group, which presumably was

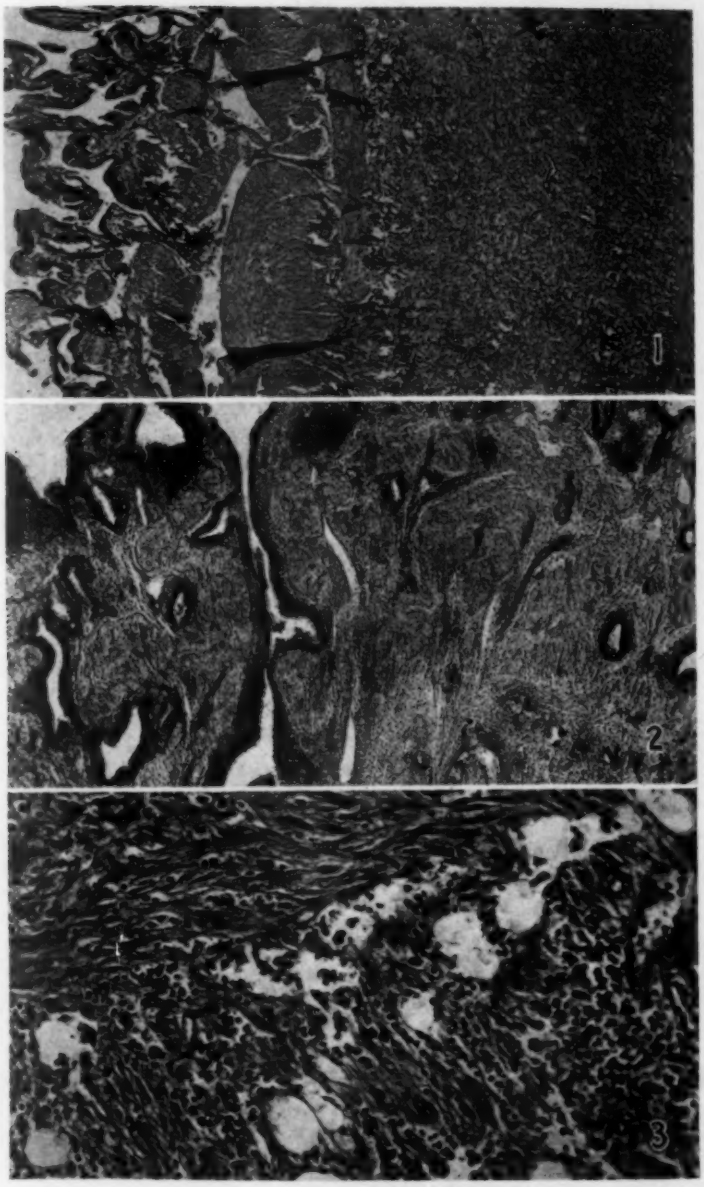

Fig. 1.—Wall of a gallbladder. Note the good preservation of the mucosal structure and the well defined hypertrophic fibromuscular layer. The subserosa is the principal site of involvement, showing a fibrotic granulomatous process in which many large vacuolated mononuclear phagocytes take part. Hematoxylin and eosin; x 26.

Fig. 2.—Wall of a gallbladder. Many glandular spaces are visible in the densely fibrotic wall. A communication between the mucosal surface and the subserosal glandular structures is demonstrated. Frozen section stained with sudan IV; x 25.

Fig. 3.—Serosa of one specimen which contains adipose tissue in which fat necrosis has occurred. Note the fibrotic changes with moderate cellular infiltration. Lymphocytes and mononuclear foam cells predominate. Hematoxylin and eosin; x 145.

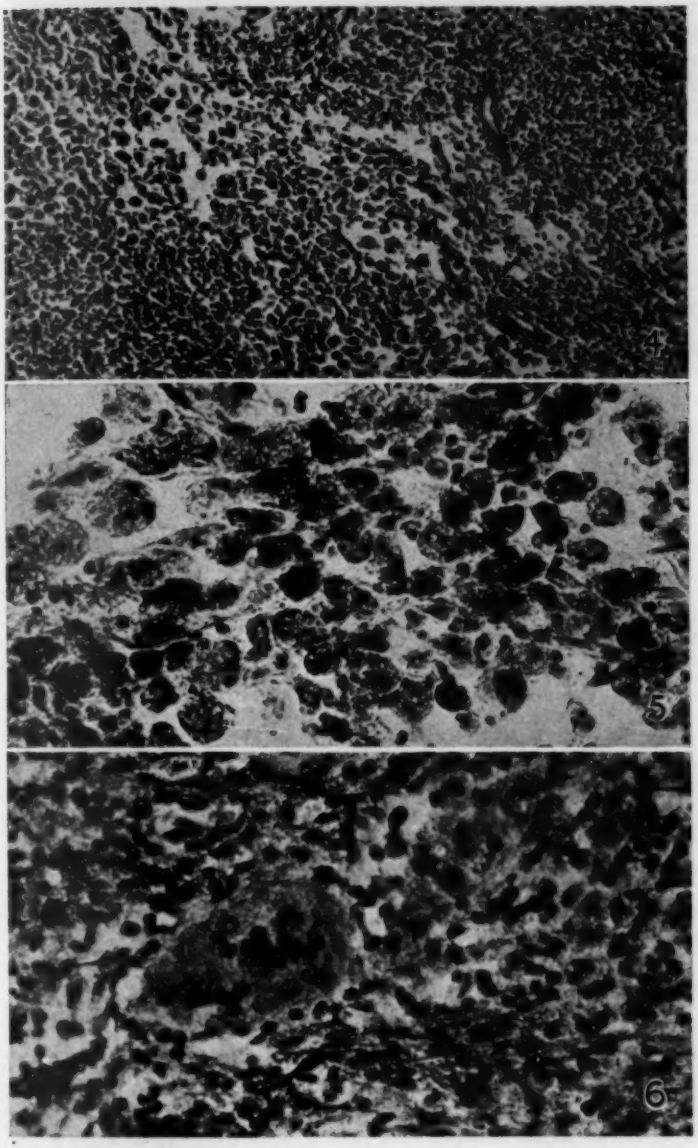

Fig. 4.—Abscess in the subserosal layer of a gallbladder. Dense cellular infiltration, edema and areas of necrosis are present. Many large mononuclear phagocytes with clear areas are visible. Hematoxylin and eosin; x 95.

Fig. 5.—Same specimen as in figure 4 showing area predominantly infiltrated by cells containing much lipid material. Hematoxylin and eosin; x 275.

Fig. 6—Wall of a gallbladder. Foam cells are packed into spaces between newly proliferated fibrous tissue. Two large foreign body giant cells, with much lipid in their cytoplasm, are present. Hematoxylin and eosin; x 350.

due to peritonitis, although no necropsy was done. In this fatal case a large, acutely inflamed, necrotic, purulent, perforated gallbladder was removed four days prior to death. At operation the patient was found to have a large fluctuant mass on the under surface of the liver, and in attempting removal the surgeon encountered a large abscess which contained stones and purulent exudate, with necrosis of the surrounding hepatic parenchyma.

The severity of inflammatory changes in the removed gallbladders as recorded during routine pathologic examination is indicated by the reports of the surgical pathologists (table 4).

Pathologic Data.—Gross Appearance: In all specimens the gallbladder wall appeared thickened to a greater or lesser extent. The wall was markedly thickened throughout in the large majority of cases, reaching in the most extreme examples a thickness of more than 2 cm. The presence of localized indurated and thickened areas was noted in the remainder of those specimens in which the depth of the wall was only moderately increased.

While necrosis and evidence of excessive dissolution of tissue could be easily identified grossly, in a few of the acutely involved areas there was no completely fragmented specimen. In only 1 case could the actual site of a gross perforation be identified. Chronic fat necrosis of the serosal surface was clearly distinguishable grossly in 1 case.

The mucosa was apparently essentially intact throughout, according to the results of gross examination, in all but 5 cases. In 1 case definite areas of ulceration were made out. In only 2 cases were the normal mucosal folds identifiable. "Strawberry" changes were grossly evident in only 1 case.

Next to the excessive thickening of the walls, the most consistent gross characteristic of these specimens was the presence of one or more areas of softening of the wall, most frequent in or about the fundus. These areas of softening, as a rule, were distinguishable because of a yellowish, yellowish green or reddish brown discoloration, in contrast to the gray fibrotic walls surrounding them. In 4 cases diffuse staining of portions or all of the wall with bile pigment was also evident.

Calculi were present in every case. In only 1 case could an impacted stone be identified in the cystic duct.

Histopathologic Appearance: Fibrosis, usually of marked intensity and frequently replacing or destroying the normal muscularis, proved in the microscopic study to be the most consistent pathologic change in these gallbladders. The subserosal or perimuscular portion of the wall was the most frequent site of the fibroblastic reaction (fig. 1), and the subepithelial portion was the least commonly involved in this change. In some cases, however, the wall was densely fibrotic, the fibrous tissue being interspersed, however, with numerous Rokitansky-Aschoff sinuses or areas of closely packed inflammatory cells or both (fig. 2). In 3 cases the fibrotic reaction involved pericholecystic adipose tissue, indicating a possible end reaction of extensive fat necrosis (fig. 3).

The mucosa was intact or partially disrupted in all but 5 specimens. In the blocks of the latter 5 specimens, the mucosa was completely lost, and extensive cellular and fibrinous exudate had replaced the mucosal lining, indicating acute ulceration. When less acute inflammatory changes were present in other portions of the gallbladder wall, the mucosa was likely to be essentially intact, and in about half of these instances some of the normal but edematous, mildly fibrotic folds were seen (fig. 1). Except in

these 5 specimens in which evidence of ulceration of the mucosa was definite, marked cellular infiltration of the subepithelial tissue was characteristically absent. A few lymphocytes, plasma cells and other mononuclear cells, as well as minimal numbers of polymorphonuclear leukocytes, were universally present. An occasional lymphoid follicle with a secondary germinal center was seen.

The muscular layer was hypertrophic in 8 specimens, normal in 6, fragmented and disrupted by necrosis and abscess formation in 4 and largely replaced by dense fibrosis in 5.

Edema, so prominently a characteristic feature of inflammation of the gallbladder, was present to a remarkable degree in 6 specimens. Fibrosis and cellular exudation were more commonly seen and tended to reduce the importance of interstitial edema present in the remainder.

Definite abscesses were clearly distinguishable in 13 of the specimens. These abscesses present a variety of cell types and, as a rule, were not well defined by typical limiting fibrous walls. Instead, they appeared as sharp areas of dissolution of the fibrotic wall of the gallbladder or as islands in a dense granulomatous inflammatory lesion. They varied in size from about a half millimeter to a full thickness of the wall of the organ.

The abscesses were most commonly seen in the subserosal layer of the gallbladder, and they intermingled with excessive fibrous tissue of that stratum, extending into the pericholecystic tissues and fat. Areas of fat necrosis could be recognized in the adjoining structures in 6 specimens, but the fat necrosis did not seem to occur in the same specimens in which abscess formation was prominent.

The cellular exudate in the sites of abscess formation was predominantly of the type characteristic of chronic inflammation. Mononuclear phagocytes or macrophages, lymphocytes and plasma cells occurred in that order of frequency (fig. 4). The granulocytic cells were present to a greater or less extent, but in only a few cases were they particularly numerous. The cytoplasm of the marcophages and other phagocytes was characteristically filled with clear areas, and in many cells the entire extranuclear cytoplasm had been dissolved away in the paraffin block preparation (figs. 4 and 5). Solid areas of physaliferous cells involving a full microscopic field, however, were seen in only 2 of the specimens (fig. 6). The fat stain studies were valuable in these instances as an aid in distinguishing solid arrangements of foam cells from extensive intracellular edema in hematoxylin-eosin sections. With sudan IV, most of the cellular areas in these specimens were strikingly filled with phagocytes which, in turn, were filled with small and large, bright red to reddish brown droplets, indicating the specificity of the fat stain for this intracellular material. In a few instances, however, the frozen sections stained with sudan IV contained many foam cells which did not take up the scarlet red dye. These cells were usually intermingled with several positively stained phagocytes. This may be interpreted as a result of a difference of lipids present, since neutral fat and cholesterol and its esters take up the scarlet red, while free fatty acids do not dissolve the dye. It is possible, therefore, that in these few cases many of the foam cells contained free fatty acid in an unemulsified form.

Of considerable interest were 4 cases in which a typical granulomatous inflammatory process was evident in the histologic study. Present, in addition to an excessive characteristic fibroblastic and capillary proliferation, in this picture were frequent multinucleated giant cells of the foreign body type and numerous "foam" cells, lymphocytes and plasma cells. In 1 case (fig. 6) this reaction occurred at the edges

of abscesses which were present throughout the serosa. The abscesses, in this case, were filled with many mononuclear phagocytes containing excessive amounts of lipids. Thus, it can be seen that a xanthomatous reaction existed as a further histopathologic variant of this peculiar inflammatory manifestation. It is doubtful that an exogenous foreign material was involved in the production of this process.

Two phenomena were observed histologically which gave some hint concerning the possible source of the lipids which were observed in the gallbladder. First, clumps of pigment were present in localized or diffuse distribution in the subserosal and muscular layers in 7 specimens. This pigment had an amorphous character and appeared to be an intense dark green. It will be recalled that in 4 of the gross specimens some bile staining of the gallbladder wall was noted. The intercellular clumps of pigment were interpreted as biliary pigments which had found their way to an intramural position. There was no way, however, to rule out the possibility of the presence of oxidized hemoglobin which had arisen from extravasated blood in the presence of a severe hemorrhagic inflammatory process.

Second, the remnants of "diverticula" or Rokitansky-Aschoff sinuses were identifiable in the blocks in 8 of the cases. In 1 case the channel by which the subserosal portion of the outpocketing communicated with the mucosal surface and the lumen of the organ was clearly demonstrated in the section studied (fig. 2). It is believed that if sufficient microscopic sections had been studied in the other cases, undoubtedly such glandular structures would have been observed in a much larger proportion.

In 1 instance definite proof was obtained that these "diverticula" played a role in the genesis of the inflammatory picture seen in many of these cases. In this instance an "abscess" was seen grossly in the wall of the fresh gallbladder. The lesion was studied carefully microscopically, and the center of the "abscess" cavity appeared to consist of thin cellular and amorphous debris, most of which fell out of the section on preparation. The "abscess" cavity was about 8 or 9 mm. in diameter and was surrounded by a dense fibrous inflammatory tissue with areas of cellular exudates containing many "foam" cells which readily stained positively with sudan IV. The real nature of the cavity was identified by the finding of strips of the remaining epithelial cells, indicating that this cavity represented an obstructed, distended diverticulum which had given rise to an intense chronic fibrous inflammatory reaction, probably as a result of the irritant effects of the imprisoned bile. Unfortunately, the lesion was not cultured prior to its contamination from pathologic study. Whether the lipoid material seen in the phagocytes surrounding this cavity was bilary in origin or was liberated from an intramural source by the cytotoxic and lipase-activating power of bile salts cannot be determined.

As was mentioned previously, fat necrosis was present both grossly and microscopically in a serosal or perimuscular location in a number of cases. Positive evidence of fat necrosis, consisting of fibrosis and cellular infiltration of areas of adipose tissue, was noted in 6 specimens. Numerous foam cells, which readily dissolved the scarlet red dye with the frozen section preparation, were present in these areas. An example of this is pictured in figure 3. In this case there was also considerable bile pigment in an intramural position, suggesting the possibility of the action of bile acids alone, or bile acids combined with pancreatic juice, as the possible irritants in this process.

Finally, not all the fat-carrying phagocytes were seen in areas of dense concentration of infiltrating cells, in areas of fat necrosis or about the Rokitansky-Aschoff sinuses. In most of these sections there were present some relatively quiescent inflammatory areas with much fibrosis and minimal cellular infiltration; yet, in the frozen

section stained with sudan IV, isolated fat-containing phagocytes were present throughout all layers. Their presence, however, would not seem significant in the absence of the other changes noted previously. In 2 cases the specially stained section revealed extensive cholesterosis which involved the mucosa and subepithelial tissue. Interestingly enough, the subserosal areas in the 2 gallbladders with cholesterosis showed only minimal numbers of phagocytes with intracellular lipids.

Chemical Data.—Cases in the Series: Chemical determinations were made in 5 of the 23 cases. In 4 of those 5 cases the mean total cholesterol content of the tissue was four to eight times that found to be present in gallbladders in which there were minimal inflammatory changes (table 5). About half of the cholesterol was in the ester form, as indicated by the results of these determinations. In 1 specimen the fatty acid fraction was below that seen in the control studies. This gallbladder had only a focalized area of extensive inflammatory change (fig. 1), while the remainder of the

TABLE 5.—*Mean Values* of Tissue Lipid Fractions Associated with Five Different Histologic Variations of Inflammatory Disease of the Gallbladder*

Histopathologic Characteristics	Cases	Cholesterol	Cholesterol Esters	Lecithin	Fatty Acid	Total Lipids
Cases in the series						
Cholecystitis with abscesses or granulomatous areas	4	0.66	0.34	2.13	5.10	5.76
Cholecystitis with extensive fat necrosis	1	0.18	0.16	2.03	17.18	17.36
Controls						
Minimal cholecystitis	2	0.08	Trace	2.14	1.70	1.78
Minimal cholecystitis with cholesterosis	4	0.19	0.11	2.18	4.69	4.88
Subacute necrotic cholecystitis	1	0.18	Trace	2.09	1.14	1.32

*The figures represent grams of the reported fraction in 100 Gm. of tissue. The analysis was done, in each case, on 1 Gm. of tissue.

gallbladder was involved in a low grade, minimal chronic catarrhal process. The results in this case suggest that the principal fractions of the lipoid material seen intramurally were free cholesterol and cholesterol esters.

In the remaining case of the 5 in which chemical determinations were made, the cholesterol content was only about twice that of the controls, but the total fatty acid content was about eight times that of the controls. It was obvious from the chemical data that this case varied considerably from the other 4, and on close microscopic study there was found to be considerable fat necrosis of the pericholecystic tissues. From these two observations, which corroborated each other, it was evident that the excessive intramural deposits of lipoid material in this gallbladder were largely on the basis of fat necrosis, and the lipids present were free or saponified fatty acids plus an undetermined amount of neutral fat (fatty acid esters). The fatty acid fraction in 3 of the 4 specimens discussed in the preceding paragraph was approximately three times the average amount found in the controls.

It is apparent that the lecithin fraction in the gallbladder does not seem to be altered by the inflammatory processes which alter the other fractions of fatty substances. In all cases the content of lecithin did not vary significantly, and no importance can be attached to the substance on the basis of this study.

Controls: Two gallbladders with minimal inflammatory changes ("nonstrawberry") and cholelithiasis and 4 gallbladders, which grossly showed minimal to moderate

degrees of cholesterosis and minimal inflammatory changes ("strawberry") with cholelithiasis were also analyzed for lipid content; for this purpose a section through the thickest portion of the wall was utilized (table 5). In the "strawberry" gallbladders the cholesterol and cholesterol ester contents were increased one and one-half to two and one-half times those of the "nonstrawberry" gallbladders. Three of the 4 specimens with cholesterosis, however, had some slight increase in fatty acid content over that of the previous group, and this may represent a slight increase of deposits of adipose tissue in the wall, which are often seen in chronic inflammation of the gallbladder (table 5).

Lastly, one acutely inflamed gallbladder, with marked edema and areas of necrosis, was chemically analyzed also. Microscopically, no remarkable intramural deposits of lipids were identified in either hematoxylin and eosin or sudan IV stained sections. The significance of slight increase in only the free cholesterol content and decrease in fatty acid content is not apparent (table 5).

Bacteriologic Data.—In 1 case tissue from the wall of a typical "abscess" filled with foam and giant cells was cultured. The result was reported as negative. In another case the records show that the content of an empyematous gallbladder was cultured and was reported positive for Streptococcus (faecalis?). No attempt was made to evaluate these limited and inconclusive data.

COMMENT

This study was undertaken to investigate an infrequently observed but significant variation in the pathologic picture of inflammatory disease of the gallbladder. It is felt that this variation should be assigned more significance in the consideration of the genesis of inflammation of the gallbladder. Pathologists have been, as a rule, reluctant to veer away from the concept of a primary bacterial infection as the basis of the pathologic changes observed. It has become increasingly evident to numerous experimental workers that the etiologic factors are other than infectious irritants in many, if not a large majority, of cases of cholecystic disease. The disappointing attempt to associate presence of pathogenic micro-organisms with severe inflammatory processes involving the gallbladder has been a disturbing factor to many in this field of investigation. Not to be discounted, however, is the observation that the cholecystitis produced experimentally by means of chemical irritants is strikingly similar to the processes seen in the human gallbladder.

The idea that all spontaneous inflammatory processes are infectious in origin has lost ground rapidly as new concepts appear to explain more adequately the clinical and pathologic phenomena. Recently it has been shown that even the presence of polymorphonuclear leukocytes, which is ordinarily reliable evidence of a serious or acute inflammatory process, does not in itself constitute evidence of inflammation of the gallbladder. McKibbin and McDonald[24] demonstrated that the role of these leukocytes was probably a metabolic one when they were present without other evidence of disease. Lymphocytes and plasma cells have

frequently been observed to be of considerable histopathologic importance in producing anatomic evidence of metabolic disturbances, as in exophthalmic goiter and plasma cell mastitis. Similarly, the presence of mononuclear phagocytes which contain excessive amounts of fatty materials in their cytoplasm has been described as important histopathologic evidence of the role of lipids in certain lesions, namely, xanthoma, "lipoid" pneumonia and fat necrosis.

These observations made on inflammatory processes in other situations in the body appear to lend weight to the idea that the finding of these phenomena in the wall of the gallbladder is important. The inflammatory processes observed in this study were characterized by three general histopathologic pictures: fat necrosis, xanthomatous reaction and necrosis with dense concentrations of infiltrating plasma cells, lymphocytes and "foam cells." Womack and Bricker[6b] stated that the xanthomatous reaction seen in the walls of diseased gallbladders, though infrequent, is probably not the result of primary bacterial action, since this reaction is never seen in locations in which bacterial infections are known to be the source of inflammation. Fat necrosis is also generally considered to be unassociated with primary bacterial activity. It is believed to result from liberation of enzymes existing locally in many, if not all, human tissues.

The question of whether the lipids which were identified provided an initial irritating effect or were liberated or were involved as a result of the action of bile, bile acids, pancreatic juice or certain pathogenic micro-organisms cannot be answered in this study.

Greater importance should be assigned to the part played by the so-called diverticula of the gallbladder. In this study concrete evidence has been presented to indicate that many intramural inflammatory processes may be evoked by irritants reaching the subserosal layer by way of these structures. They may provide an explanation of certain cases of bile peritonitis without demonstrable perforation of the gallbladder.[25] Shepard,[26] in his thesis on benign neoplasms of the gallbladder, reported that he found 14 per cent of the adenomas associated with actual abscess formation and that all were characterized by chronic inflammatory reaction in the surrounding wall.

Cholesterol, cholesterol esters and fatty acids are the important lipid fractions occurring in excess in the walls of inflamed gallbladders which show lipid deposits. The cholesterol compounds are present in

24. McKibbin, J. P., and McDonald, J. R.: Surgery **17**:319, 1945.

25. Buchanan, J. J.: Surg., Gynec. & Obst. **26**:303, 1918.

26. Shepard, V. D.: Benign Neoplasms of the Gallbladder, Thesis, University of Minnesota Graduate School, 1941.

cholecystic bile in considerable concentration, and it is likely that these substances reached their intramural location in these cases by infiltrating from the lumens of the diverticular structures present. The marked increase in the fatty acid fraction in one specimen associated with histologic evidence of fat necrosis indicates that fatty compounds can be liberated locally as a result of enzyme activity.

It will be seen, therefore, that certain practical clinical considerations may result from further investigation of the etiologic role of chemical irritants in cholecystic disease. The concept that infection plays a minor or insignificant role will lead to a thorough revision of many therapeutic principles in the management of cholecystitis.

SUMMARY AND CONCLUSIONS

A study of the clinical records and a gross and microscopic investigation of the gallbladder, combined with chemical determination of the lipid fractions present in the tissues, were carried out in 23 cases of advanced inflammatory disease of the gallbladder. Complete clinical and pathologic data are presented in all cases, while results of the determination of tissue lipid fractions are recorded for 5 of the 23 specimens. For control data, chemical studies were made of 7 additional fresh specimens.

The presence of advanced, complicated, inflammatory lesions of the gallbladder in the 23 selected cases was not associated preoperatively with corresponding clinical evidence of a severe infection or a purulent intra-abdominal disorder. Furthermore, except for the one postoperative death, infection or sepsis did not complicate the recovery from the surgical procedures performed in the treatment of the disease.

The local lipid disturbances in the walls of the gallbladder were associated with active perforating purulent lesions, but there was no clinical evidence of significant bacterial activity such as is usually seen in similar lesions which involve other intra-abdominal viscera—for example, the appendix. Obstruction of the cystic duct with resultant stasis or imprisonment of bile occurred in most, if not all, of these cases.

In addition to many of the usual histopathologic changes seen in inflammation of the gallbladder, the 23 specimens revealed evidence of excessive deposits of lipids in either an intramural or a pericholecystic location. For the most part, the lipids were in the form of deposits occurring in large mononuclear phagocytes or giant cells. The lipids were associated with three types of histopathologic picture, namely, a xanthomatous granulomatous reaction, fat necrosis and intramural areas of dense cellular infiltration with necrosis and "abscess" formation. The histopathologic evidence presented, suggestive of a chemical origin of the cholecystitis seen in these patients, would explain the paucity or absence of clinical signs of infection or sepsis.

Chemical determinations of lipid fractions of the tissues from 5 of the gallbladders indicated that the cholesterol and the cholesterol esters were significantly increased in those specimens that showed histopathologic evidence of excessive deposits of fatty substances. When fat necrosis predominated, there was a marked increase in the fatty acid fraction. When the other characteristic lipid lesions were seen, the fatty acid fraction was only slightly increased.

Rokitansky-Aschoff sinuses or diverticula of the gallbladder appeared to play an important role in the production of this type of inflammatory reaction. These frequently occurring structures in the wall of the gallbladder may explain the aforementioned and other unusual features of cholecystic disease.

The phenomena observed suggest that chemical irritants or metabolic disturbances play a major role in the development of many of the inflammatory processes of the gallbladder.

CONGENITAL CYSTS OF THE LUNG

Report of a Case with Successful Pneumonectomy

A. C. WEBB, M.D.

Pathologist to Provident Hospital

CHICAGO

THE FIRST CASE of congenital cystic disease of the lung in American literature was reported by Koontz[1] in 1925. He reviewed all cases that he could find in the literature. The total number was 108, nearly all of which were in the German literature; only a few were in the English, French and Italian. Since these cases included several allied conditions, such as diverticula of the trachea and main bronchi, solitary cysts of the tracheal and bronchial walls, cysts of aberrant lung tissue and of accessory lobes, Koontz concluded that the total number of true congenital cysts was considerably less than 100.

In 1937 Rabinowitz and Rogers[2] found less than 300 cases of cyst of the lung in the literature. In the same year Schenck[3] collected 381 cases. In 1946 Adams, Phillips and Hanni[4] wrote a paper on the differential diagnosis and pathologic classification of 21 cases they had studied. The most recent contribution is that of Norris and Tyson,[5] who reported 2 cases under the title "Congenital Polycystic Lung Disease" and called attention to the correlation of this and polycystic disease of other epithelial organs. Several reports of individual cases have been made, but the number of recorded cases of true congenital cyst is not known because some authors do not differentiate the acquired and congenital forms. The term "cystic disease of the lung" is defined by Klosk, Bernstein and Parsonnet[6] as any condition in which the lung parenchyma is replaced by sharply defined cavities containing fluid or air. Other terms applied to this condition are "honeycombed lung," "fetal bronchiectasis," "cystic bronchiectasis," "cystic pulmonary disease," "pneumatocele" and "pneumatocyst."

The true congenital cyst is unilocular or multilocular and is characterized histologically by a lining of ciliated columnar or cuboidal

---

1. Koontz, A. R.: Bull. Johns Hopkins Hosp. **37**:340, 1925.
2. Rabinowitz, L., and Rogers, E. J.: New England J. Med. **216**:919, 1937.
3. Schenck, S. G.: Arch. Int. Med. **60**:1, 1937.
4. Adams, W. E.; Phillips, F. J., and Hanni, J. W.: Iowa M. Soc. **63**:425 and 433, 1946.
5. Norris, R. F., and Tyson, R. M.: Am. J. Path. **23**:1075, 1947.
6. Klosk, E.; Bernstein, A., and Parsonnet, A. E.: Ann. Int. Med. **24**:217, 1946.

epithelial cells and a wall in which may be found mucous glands, cartilage and concentric bundles of smooth muscles. It is usually limited to one lung but is occasionally bilateral, and it has been found in the fetus, in the newly born and in later life at all ages. Secondary infection is frequent, but tuberculosis is not common: in only 5 of the cases collected by Koontz had tuberculosis developed. According to Sharpe,[7] centuries before the advent of roentgenology this disease was known as vesicular emphysema or hypertrophic pulmonary emphysema.

The pathogenesis of the congenital form has been explained by many authors, and many different theories have been proposed. The most plausible theory asserts that the bronchiectasis is due to aplasia of the alveoli. In the fourth week of embryonic life the lungs appear as buds, the ends of which are lobulated. The terminal portions of the lobules branch to form atriums, and at about the sixth month the alveoli are formed from evaginations of the atriums. If this development is arrested prematurely, the ends of the already formed bronchi will dilate and form cysts that take the place of the alveoli.[8] Although the cause of the aplasia is not known, the standard textbooks of developmental anatomy tend to support this theory. According to Arey,[9] early in the sixth month, the terminal buds have the appearance of irregular spaces bordered by capillary networks, and this is the permanent structure of the alveolar sacs. These evaginations of the terminal air passages do not have the characteristic cuboidal cell epithelium of the bronchial buds. Therefore, the presence of lung cysts lined with respiratory epithelium indicates aplasia or early complete regression of alveoli.

The acquired form of the disease is characterized histologically by the presence of coal pigment in the contiguous alveolar walls and by blebs and bullae at the periphery of the lung. It is associated with any condition that may cause incomplete bronchial obstruction, such as presence of a foreign body, infection of the respiratory tract, chronic bronchitis, bronchial asthma, pulmonary fibrosis and emphysema.[4]

The roentgenographic diagnosis of the congenital form is based on the honeycombed appearance of the pulmonic field, described by Wood[10] as "oval or smoothly rounded fine annular shadows of almost

---

7. Sharpe, C. T.; Radiology **34**:692, 1940.

8. Kaufmann, E.: Lehrbuch der pathologischen Anatomie, translated by S. P. Reimann, Philadelphia, P. Blaikston's Son & Company, 1929, vol. 1, pp. 335-356.

9. Arey, L. B.: Developmental Anatomy, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 231.

10. Wood, W. B.: Proc. Roy. Soc. Med. **33**:335, 1940.

distinctive appearance before lipiodol." When diagnosis was based on roentgenologic evidence alone, in many cases, according to Sante,[11] cyst of the lung was diagnosed as congenital without adequate proof. Pierce[12] stated emphatically that congenital pulmonary cyst is a rare lesion and must be roentgenologically evident from birth and must be histologically proved. Other writers have stated that the presence of bronchial structures positively proves that the cysts are congenital in origin.

REPORT OF A CASE

The medical history of the patient, a girl 12 years of age, began in the Cook County Hospital, May 22, 1945. She had a cough of a duration of one year, early fatigue and swollen veins of the legs for four weeks and a loss of weight of 7 pounds (3 Kg.) in one year. With injection of iodized poppyseed oil 40 per cent, numerous bronchiectatic cavities were demonstrated throughout the entire left lung. The heart was drawn to the left, and the ribs on this side were retracted. Microscopic examination of fluid from the left bronchus revealed fibrin, red blood cells and a few round cells. The Kahn and Wassermann tests were negative. A diagnosis of broncheictasis was made with the supposition that the primary etiologic event was the lodging of a foreign body in the bronchus, with resultant development of bronchiectatic cavities and an abscess.

In May 1946 the patient was admitted to Provident Hospital because of a cough, productive of grayish yellow, foul-smelling sputum, and pain in the left side of the chest. Other complaints were vomiting, loss of weight and general weakness. At birth she weighed 9½ pounds (4, 309 Gm.). Measles and mumps were the only previous diseases. She lived with her mother and eleven siblings, and the family were clients of the County Bureau of Public Welfare.

On admission her temperature was 99, pulse rate 108 and respirations 48. She weighed 61 pounds (27.5 Kg.) She was poorly developed and poorly nourished but not acutely ill. The significant physical findings were in the left side of the chest. These were diminished expansion, absence of vocal fremitus, absence of tactile fremitus and dulness over the entire hemithorax.

The roentgen diagnosis (Dr. William P. Quinn) was atelectasis, bronchiectasis and congenital cystic disease of the left lung (fig. 1*A*). Bronchoscopic examination (Dr. Harold W. Anderson) revealed free flowing, odoriferous, grayish yellow purulent fluid in the left bronchus but no obstructing lesion.

No acid-fast bacilli were found in the sputum after repeated examinations. Two red blood cell counts were recorded: One was 3,850,000, with a hemoglobin content of 10 Gm., and the other was 4,400,000, with a hemoglobin content of 12.5 Gm. The white blood cell counts varied from 9,750 to 12,000. The other laboratory findings were not significant.

The patient continued to expectorate large amounts of foul-smelling sputum. Her temperature varied from 98 to a high point of 104F., but the usual elevation was 99.

External pulmonary drainage and pneumonectomy were considered after extended administration of sulfadiazine and penicillin had produced no improvement. On June 13, total left pneumonectomy was performed by Drs. M. M. Shaw and J. E. Bryant. The postoperative course was uneventful, and the patient was discharged from the hospital on the twelfth postoperative day.

---

11. Sante, L. R.: Radiology **33**:152, 1939.
12. Pierce, C. B.: Am. J. Roentgenol. **44**:848, 1940.

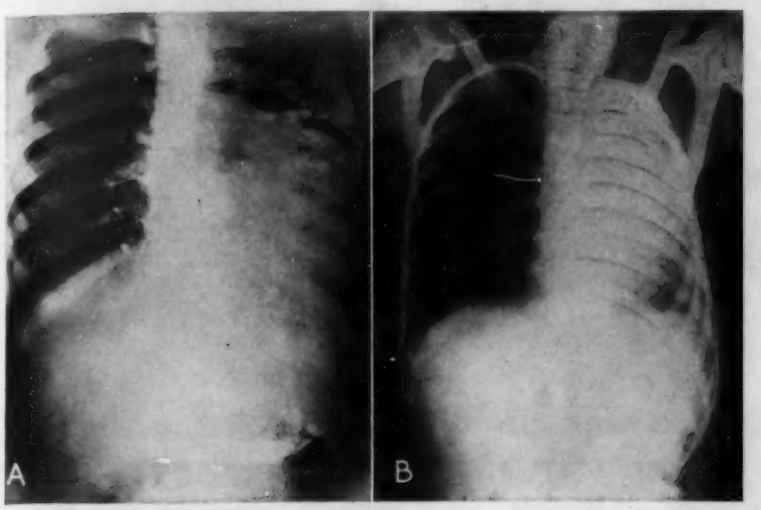

Fig. 1.—*A*, roentgenogram of the chest made after insufflation of an opaque medium. Almost complete basal opacity appears on the left. Patchy aeration is seen. Note sacculation of the contrast medium with some fluid levels. The right lung shows compensatory emphysema.

B, postoperative roentgenogram of the chest showing mediastinal shift to the left, increased density of the left hemithorax, partial regeneration of the ribs and slight scoliosis of the dorsal part of the spinal column.

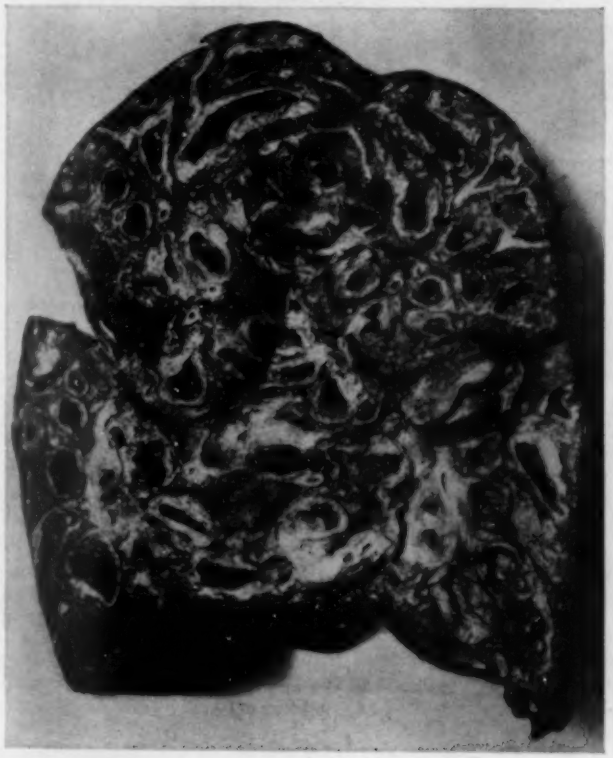

Fig. 2.—Photograph of the lung showing the sectioned surfaces. The numerous dilated bronchi contained purulent exudate.

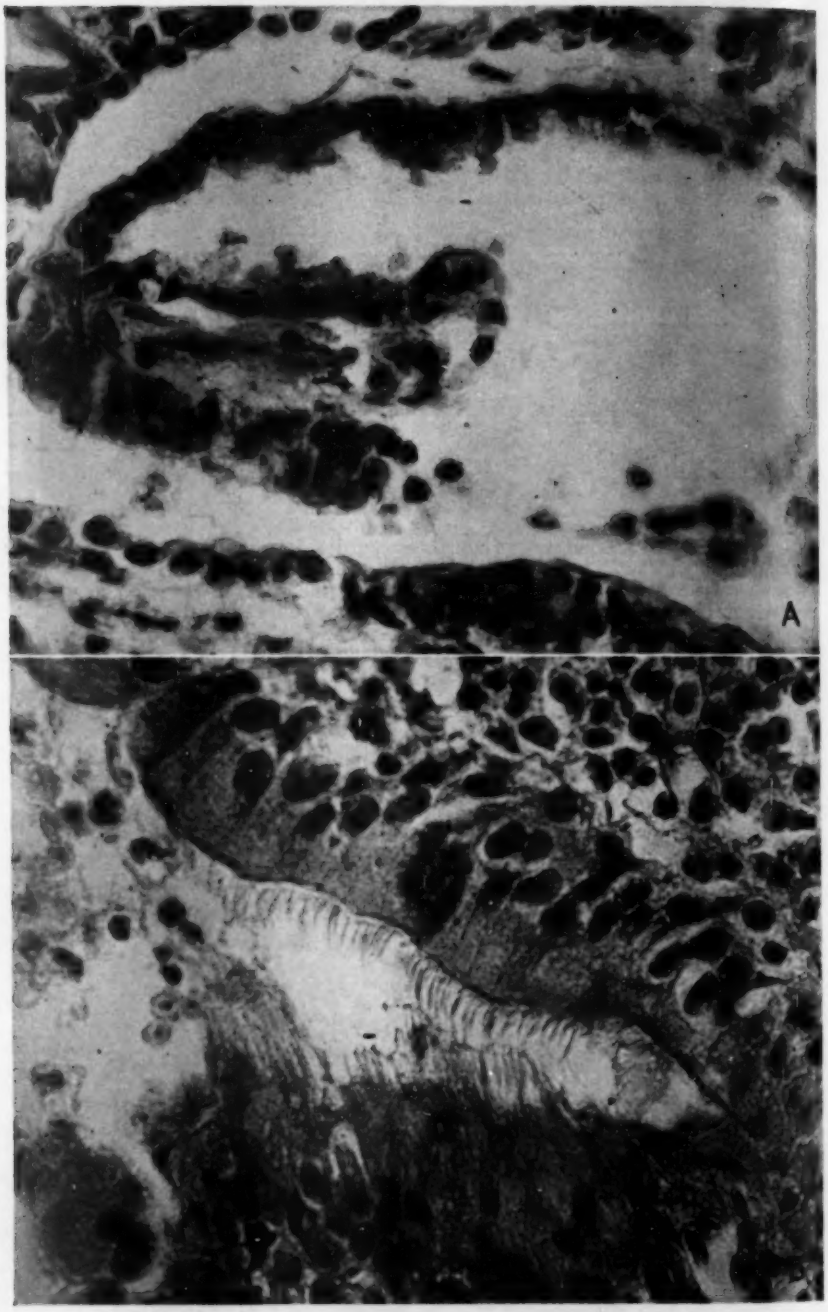

Fig. 3.—*A*, section of a cyst, showing the cuboidal and columnar epithelial cell lining. Hematoxylin and eosin; x 3,800; oil immersion.

B, section showing the ciliated, pseudostratified columnar cell epithelium of a cyst and the inflammatory exudate in the lumen. Hematoxylin and eosin; x 3,800; oil immersion.

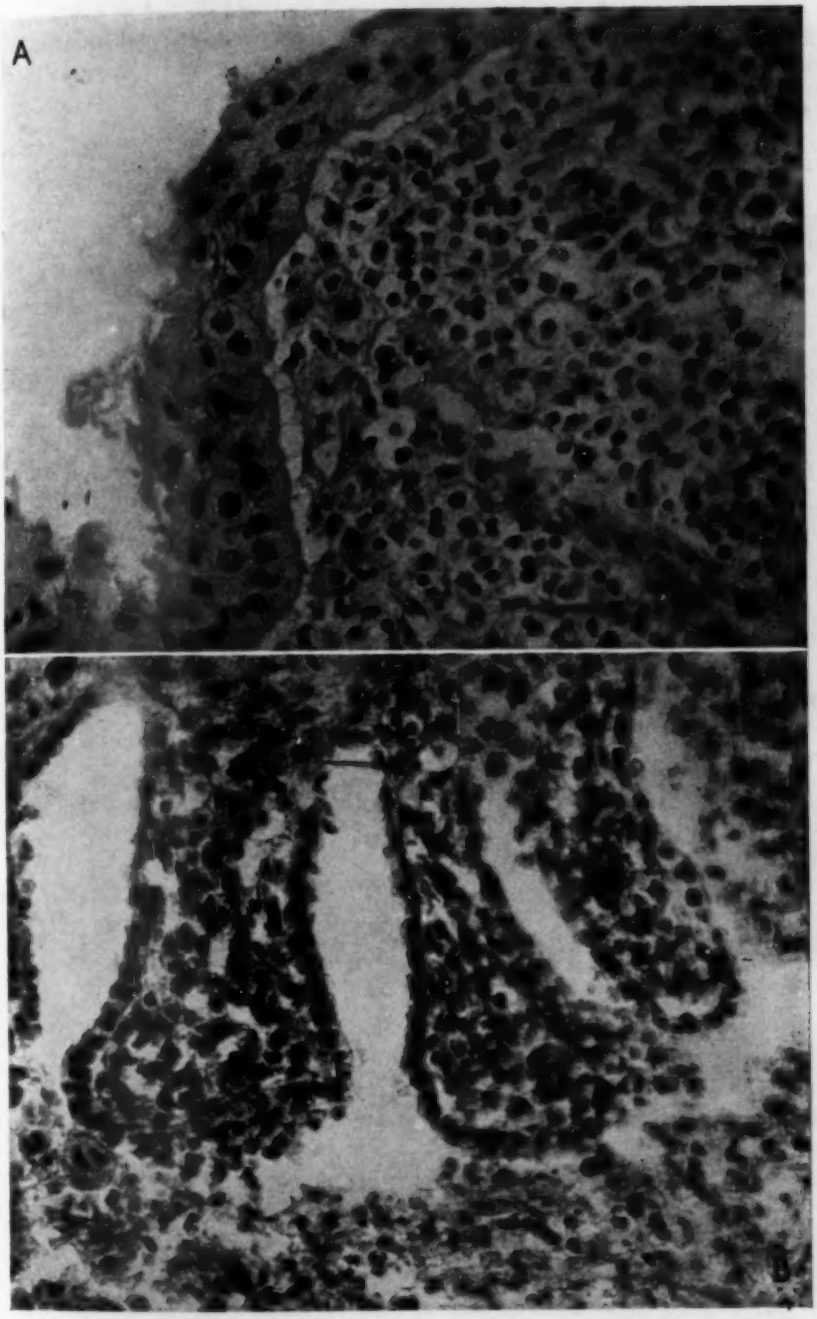

Fig. 4.—*A*, section showing squamous metaplasia of the respiratory epithelium and infiltration of the wall with chronic inflammatory cells. Hematoxylin and eosin; x 1,760. *B*, papillary projections of columnar cell epithelium in dilated bronchi. The lumen contains inflammatory cells and cellular debris. Hematoxylin and eosin; x 1,760.

Six months later, her weight was 71 pounds (32 Kg.), and she had no complaints. A roentgen examination in January 1947 showed almost complete mediastinal shift to the left, partial regeneration of the resected fourth rib and complete opacity of the left lung field (fig. 1*B*).

Gross Anatomic Description of the Specimen.—The specimen, received fresh, weighed 345 Gm. (fig. 2). It was a left lung, the external surface of which had several scattered tough fibrous tags. When it was sectioned, a large amount of thick grayish pus escaped. The sections showed numerous round or oval cavities that varied in diameter from 2 mm. to 2 cm. Probing disclosed that several of these cavities communicated with the main bronchus. Their walls were grayish yellow and firm in consistency. They were from 1 to 2 mm. thick. The cavities contained thick purulent fluid, and their inner surfaces were grayish to dark red. The thin bands of tissue between the cavities were dark reddish gray and firm in consistency. Clustered around the main bronchus were several firm nodes that varied in diameter from 7 mm. to 3 cm. There was also a section of rib, 17 cm. long with a breadth of 10 to 15 mm.

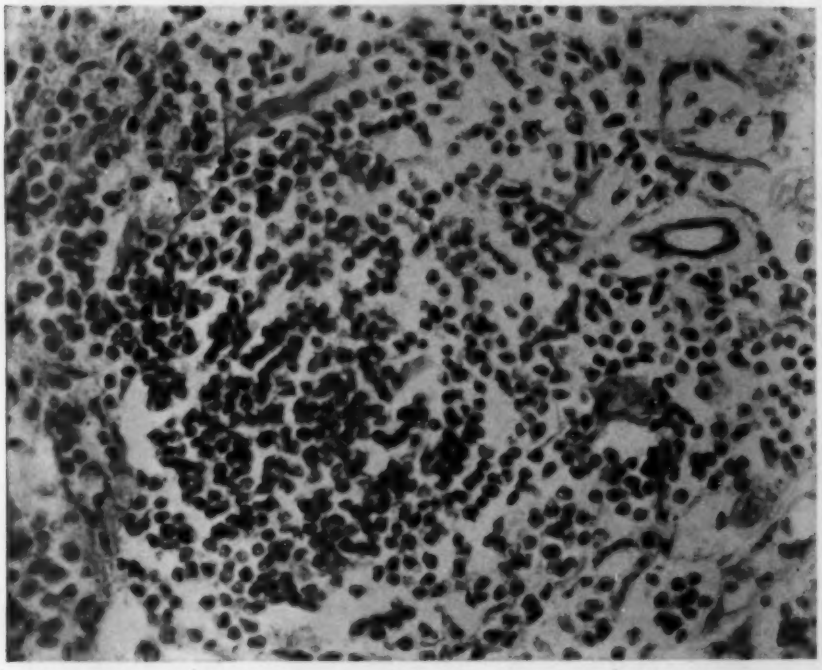

Fig. 5.—Section showing the tissue between the cysts infiltrated by lymphocytes, mononuclears, plasma cells and neutrophilic polymorphonuclear leukocytes. Hematoxylin and eosin; x 1,760.

Histologic Description.—The microscopic sections showed cysts of various sizes lined with cuboidal or columnar epithelial cells arranged in one or more layers (fig. 3*A*). Many cysts showed characteristic ciliated, pseudostratified columnar cell epithelium (fig. 3*B*). A cellular exudate was adherent to the walls. In some fields the wall was

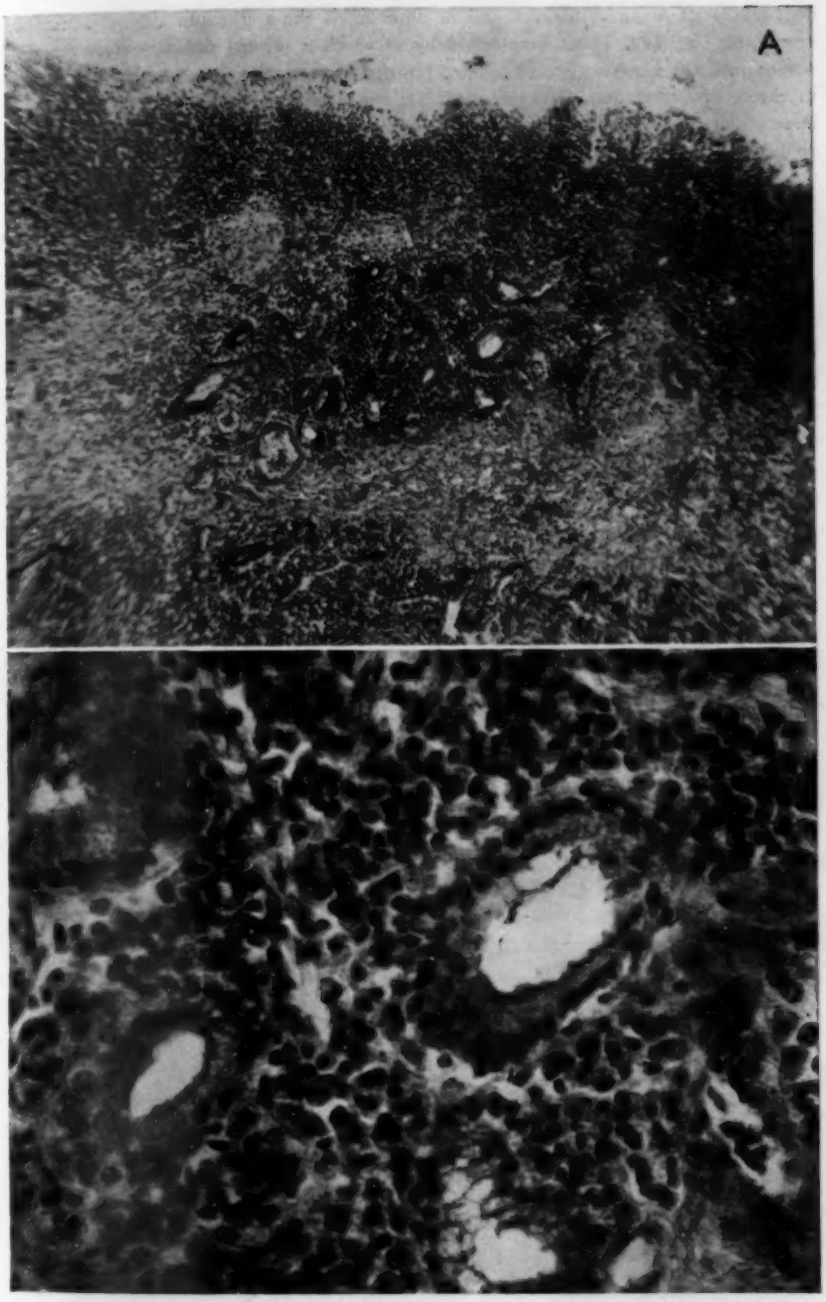

Fig. 6.—*A*, section showing the cyst wall heavily infiltrated with inflammatory cells. Note mucous glands and proliferation of fibrous connective tissue. Hematoxylin and eosin; x 400.

B, higher magnification of a part of *A*, to show details of the mucous glands. Hematoxylin and eosin; x 1,760.

denuded of its lining epithelium and in other fields the epithelium showed squamous metaplasia (fig. 4*A*). There were also fields in which it formed papillary folds that extended into the lumens (fig. 4*B*). The epithelial proliferation was probably secondary to chronic inflammation. Between the cysts were collagenous fibers and an extensive infiltrate of lymphocytes, mononuclears, plasma cells and a few eosinophils, also hemorrhage, deposits of hemosiderin and miliary abscess (fig. 5). A few mucous glands were seen in the walls of the cysts (fig. 6). The lymph nodes showed chronic inflammatory hyperplasia.

SUMMARY

Cystic disease of the lung may occur in both the acquired and the congenital form. A case of the congenital form with purulent infection is reported. The patient was a girl of 12 years. Total pneumonectomy was performed, from which she made an uncomplicated recovery. Pneumonectomy was successfully performed for this disease in an infant 23 days old by Gross.[13] A critical review of the literature indicates that true congenital cyst of the lung is rare.

13. Gross, R. E.: Ann. Surg. **123**:229, 1946.

CRANIOLACUNIA

Report of Six Cases, with a Review of the Literature

J. P. WYATT, M.D.
AND
H. GOLDENBERG, M.D.
TORONTO, CANADA

CRANIOLACUNIA is an abnormality affecting fetal skulls and is characterized by a variable netlike or arborizing pattern of bony ridges, which sharply delineate and separate small rounded depressions or defects on the inner surface of the bones of the cranial vault. It is usually associated with spina bifida and the latter's related neuropathologic variants.

There are a number of synonyms for the condition. Writing from Germany years ago, Engstler[1] gave this phenomenon the popular name of *Luckenschadel* (breached skull). Vogt and Wyatt[2] have preferred the title "craniolacunia." "Relief skull," "trabecular skull," "lacunar skull," and "cranial lacunous osteogenesis" are other designations. In the German iterature, in addition to Engstler's term, this cranial bone fault is known as *Blasenschadel* (bubble skull) and *Leistenschadel.*

West[3] is usually credited with having reported the first case in 1875; actually Hoffman[4] had published a study of the condition in the previous year. In 1886 White[5] reported a case of "trabeculated skull", and in the same year von Recklinghausen[6] added 2 cases, drawing attention to the associated spina bifida. Engstler in 1905 collected 11 cases from the literature and added one of his own. Wieland[7] in 1909 reviewed the subject and supported the etiologic theory of increased pressure. Hughes[8] in 1921 confused this condition with craniotabes. In

---

From the Department of Pathology, Toronto East General Hospital.

1. Engstler, G.: Arch. f. Kinderh. **40**:322, 1905.
2. Vogt, E. G., and Wyatt, G. M.: Radiology **36**:147, 1941.
3. West, J.: Lancet **2**:522, 1875.
4. Hoffman: Vrtljrschr. f. d. prakt. Heilk. **122**:53, 1874.
5. White, cited by Karshner and Reeves.[10]
6. von Recklinghausen, F.: Virchows Arch. f. path. Anat. **105**:243, 1886.
7. Wieland, E.: Virchows Arch. f. path. Anat. **197**:167, 1909; Cor.-Bl. f. schweiz. Aertze **39**:588, 1909.
8. Hughes, E.: Lancet **2**:1045, 1921.

1924 Cohn[9] discovered craniolacunia in otherwise normal children. The subject was introduced into the American literature in 1907 by Markoe.[10] Then followed a gap of twenty-six years before the subsequent publications of Kerr,[11] Doub and Danzer,[12] and Maier[13] appeared. These authors summarized the previous literature and added their own observations. Rothbart[14] in 1936 brought forward 8 additional cases. Dorrance[15] in 1940 described the condition as observed in a 17 year old patient without spina bifida.

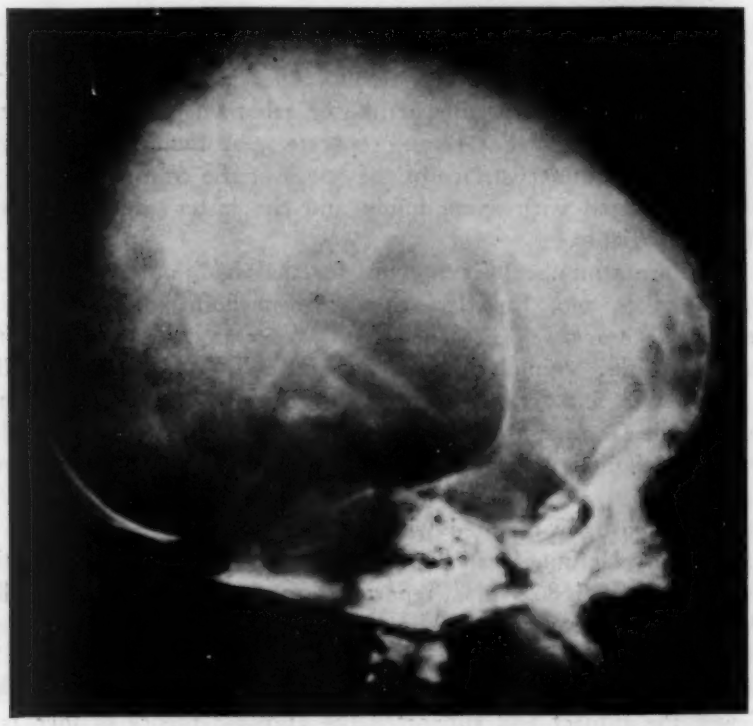

Fig. 1.—Postmortem roentgenogram showing pronounced lacunar formation of the frontal bones, with hydrocephalus.

Vogt and Wyatt made roentgenologic examinations of 6,000 skulls of infants and found that craniolacunia was present in 43 per cent of

9. Cohn, M.: Jahrb. f. Kinderh. **56**:333, 1924.
10. Markoe, J. W.: Bull. Lying-in Hosp. **4**:1, 1907.
11. Kerr, H. D.: Am. J. Roentgenol. **30**:458, 1933.
12. Doub, H. P., and Danzer, J. T.: Radiology **22**:532, 1934.
13. Maier, R. J.: Radiology **23**:615, 1934.
14. Rothbart, H. B.: Am. J. Dis. Child. **52**:1375, 1936.
15. Dorrance, T. O.: Am. J. Dis. Child. **60**:359, 1940.

120 cases of meningocele. This mass investigation revealed that in only 2 cases was craniolacunia not associated with meningocele. In 1943 Ingraham and Scott[16] reported that the majority of their 20 patients with the Arnold-Chiari malformation showed craniolacunia. An incidence of nearly 1 per cent of all births was reported by Hartley and Burnett[17] in England, in 1944. In one year, by roentgenologic examination, they encountered craniolacunia in 28 of 3,828 newborn infants. Following this up, they found that antenatal roentgenologic examination of 232 pregnant women revealed an incidence of craniolacunia of 4.3 per cent (fig. 1).

The fact that in this hospital craniolacunia has been observed at autopsy in 6 of 62 stillborn and neonatal infants in one year warrants further attention being drawn to this anomaly-complex. In this same period there was a total of 2,757 deliveries. In this group of 62 infants on whom autopsies were made there were 24 with major embryologic defects. Four of the 6 infants found to have craniolacunia were stillborn. All of the 6 infants were girls.

REPORT OF CASES

CASE 1.—A girl was born to a 34 year old primipara after thirty-seven weeks' gestation. Labor started spontaneously and lasted thirty hours. The presentation was a footling and the delivery manual. The respirations and the color were only fair, and the infant lived four hours. The health of both parents was apparently good. The complement fixation test of the mother's serum for syphilis was negative.

Anatomic Diagnosis.—Craniolacunia, hydrocephalus, spina bifida (lumbar), meningomyelocele, talipes (bilateral), microgyria, subgaleal hemorrhage.

Autopsy revealed fairly extensive craniolacunia with many complete perforations. The condition involved all the bones of the vault of the skull. Beneath one of the perforations there was a tear in the dura and the cerebral cortex which allowed cerebrospinal fluid to accumulate beneath the scalp.

CASE 2.—A hydrocephalic stillborn girl was delivered to a 27 year old woman after forty weeks of gestation. Labor started spontaneously and lasted thirty-six hours. The fetal position was a persistent posterior, complicated by the large head. Delivery was by midforceps, and the fetus was stillborn. The mother had had two normal pregnancies, and the health of both parents was apparently good. The Wassermann test of the mother's serum revealed no syphilis.

Anatomic Diagnosis.—Craniolacunia, hydrocephalus, spina bifida (lumbar), meningomyelocele, subgaleal hemorrhage.

At autopsy craniolacunia involving all the bones of the vault was observed, associated with hydrocephalus and meningomyelocele.

CASE 3.—A hydrocephalic stillborn girl was delivered to a 31 year old woman who had had three pregnancies with normal live births. This fetus was full term and a breech presentation. Labor started spontaneously and lasted three hours. Delivery was complicated by a prolapsed cord and by the breech presentation. The fetus was stillborn. The Wassermann test of the mother's serum was negative.

---

16. Ingraham, F. D., and Scott, H. W., Jr.: New England J. Med. **229**:108, 1943.
17. Hartley, J. B., and Burnett, C.W.F.: Brit. J. Radiol. **17**:110, 1944.

Anatomic Diagnosis.—Craniolacunia, hydrocephalus, spina bifida (thoracic), meningomyelocele, petechiae of the pleura and the pericardium, accessory spleen.

The postmortem examination revealed moderate craniolacunia with a few perforations, restricted to the vault.

CASE 4.—A hydrocephalic stillborn girl was delivered to a 22 year old multipara who had two live-born infants, the second of which had spina bifida and lived for three weeks. The health of both parents was apparently good. Gestation lasted forty weeks. The presentation was vertex, and delivery was accomplished after version on account of the hydrocephalic head. The Wassermann test of the mother's serum was negative.

Anatomic Diagnosis.—Hydrocephalus, spina bifida (thoracolumbar), meningomyelocele, craniolacunia, ependymal granulomatosis of the lateral ventricles of the brain, coccygeal dimple.

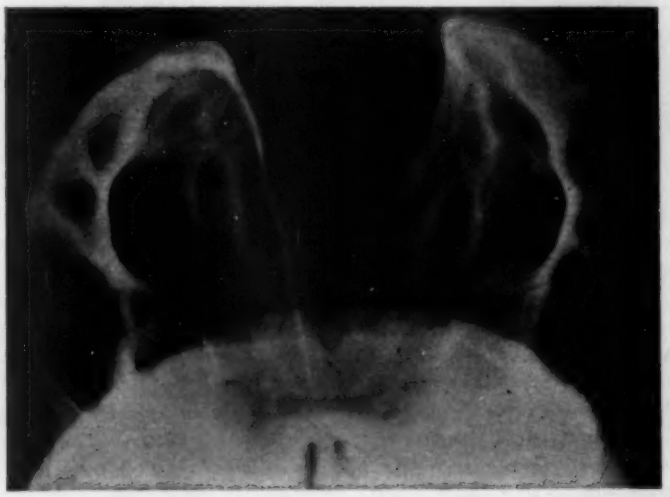

Fig. 2.—Postmortem roentgenogram demonstrating craniolacunia of the frontal bones.

This stillborn infant had marked craniolacunia in the frontal and parietal regions. The base of the skull showed marked ridging, limited principally to the middle cranial fossa.

CASE 5.—A hydrocephalic girl was delivered to a 23 year old primipara after 35 weeks' gestation. Labor started spontaneously and lasted two hours. The presentation was breech, and the delivery was complicated by a prolapsed cord. The fetus was stillborn. The health of both parents was apparently good. The Wassermann test of the mother's serum was negative.

Anatomic Diagnosis.—Craniolacunia, hydocephalus, spina bifida (lumbar), meningomyelocele, talipes (bilateral), lumbar kyphosis, postanal dimple, fractures of the femurs and the tibias, subgaleal hemorrhage, petechiae of the pericardium, the lower part of the trunk and the limbs.

In this stillborn infant, craniolacunia was most pronounced in the frontal bones. The floor of the skull was involved. The fractures were traumatic in origin, as the delivery was of a difficult breech type (fig. 2).

CASE 6.—A girl was delivered to a 27 year old multipara who had had three pregnancies. The first was complicated by difficult labor because of a contracted pelvis, with the result that the following two pregnancies were terminated by cesarian section. The present period of gestation lasted forty weeks. Labor started spontaneously and lasted four hours. Delivery was by cesarian section, and the infant lived three days. The mother's blood contained Rh agglutinogen. The Wassermann test of her blood revealed no syphilis. During her fourth month of pregnancy, the mother was exposed to measles when her three children contracted the disease. The distinction between simple measles and rubella was not made.

Anatomic Diagnosis.—Meningoencephalocele, cranium bifidum (occipital), spina bifida (cervicothoracic), craniolacunia, Klippel-Feil syndrome with absence of four cervical vertebrae, hydrocephalus, Arnold-Chiari malformation, coccygeal dimple, petechial hemorrhages in lungs, pleura and pericardium.

The autopsy on this neonatal infant revealed craniolacunia in all the cranial bones of the vault, associated with numerous other congenital anomalies.

PATHOLOGY

Craniolacunia is usually, but not invariably, associated with spina bifida or cranium bifidum, meningocele, meningomyelocele or encephalocele. Hydrocephalus occurs with it frequently. In all the 6 cases reported from this hospital craniolacunia was associated with hydrocephalus and overt defects of brain and cord. Other anomalies which have been found with craniolacunia are craniostenosis, talipes, defective formation of ribs, maldevelopment of the face, cleft palate, mongoloid facies and microcephalus. In one of von Recklinghausen's cases, congenital absence of the radius and deformity of the humerus were present. In Karshner and Reeve's[18] cases, porencephaly, congenital heart disease, arthrogryposis and micrognathia were demonstrated. In case 6 reported in the foregoing section of this article, the Klippel-Feil syndrome and the Arnold-Chiari malformation were associated with the lacunar condition of the skull.

The gross pathologic changes of the cranial vault were similar in all 6 cases, varying only in degree and extent.

The lacunas or perforations were found in the developing membranous bones of the skull, that is, the frontal, parietal, squamous temporal and occipital bones. The cartilaginous bones were free of pathologic defects. The clavicle, the classic seat of membranous ossification, was constantly free of any osseous maldevelopment.

In 6 cases dense bony bars or ridges enclosed and separated the oval depressions or defects of the inner surface of the cranial bones.

The defects in some regions were completely devoid of bony structure, and in these cases the lacunas were bridged by membranous diaphragms of periosteum and dura mater. A few of the defects had a thin additional covering of bone which corresponded to the outer table of the skull, the inner table being incomplete. The defects extended, therefore, from the inner to the outer surface.

---

18. Karshner, R. G., and Reeves, D. L.: Am. J. Roentgenol. **57**:321, 1947.

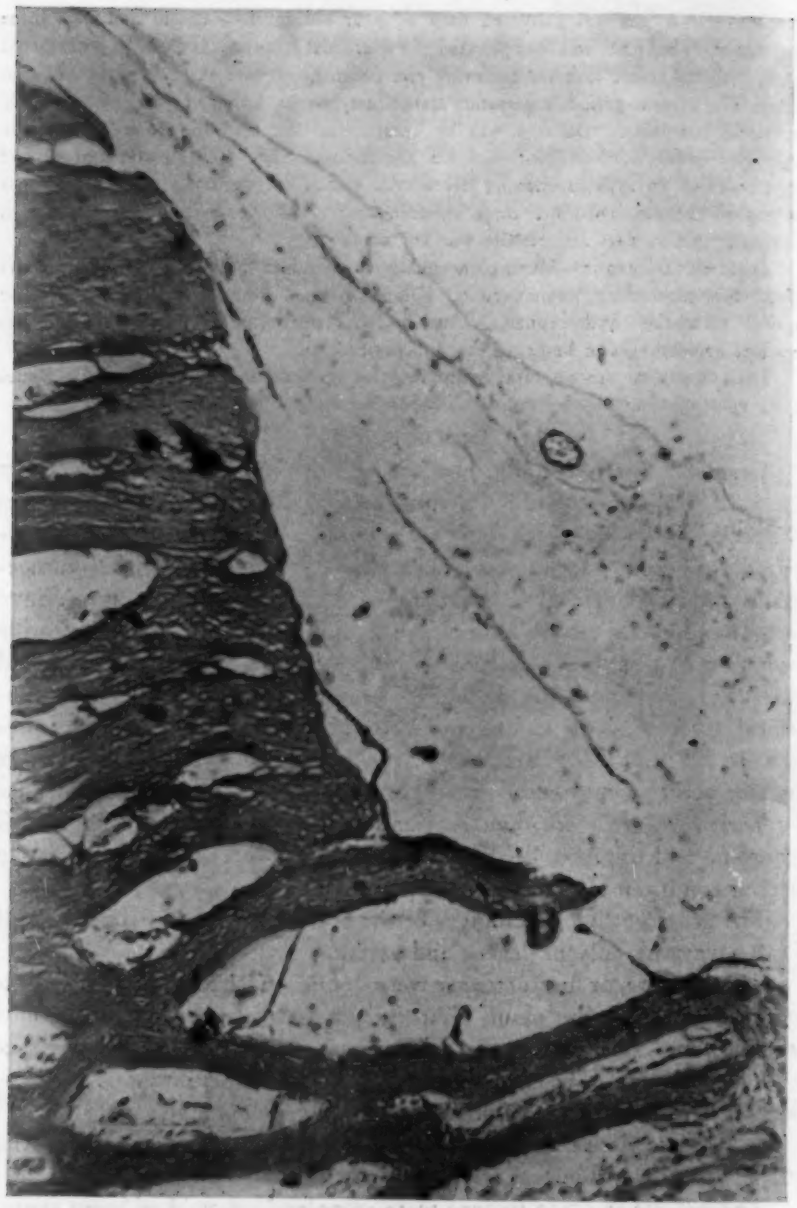

Fig. 3.—Incline of the lamellar ridge bone of the inner table; x 200. This type of bone separates the lacunas.

The bony ridges were either broad or narrow, while the lacunas between them were either shallow and poorly defined or deep; in some regions there were complete perforations. For these perforations Hartley and Burnett[19] used the term "craniofenestria" as a substitute for "craniolacunia," although there is no doubt that the difference is one of degree and that the perforations are not a separate autochthonous process. In the vicinity of the sutures there were thinning and shelving, leaving serrate indentations. This process was most marked in the cases of advanced craniolacunia.

The defects varied in size, number and shape, some being large enough for the cranial contents to bulge through them. All the bones of the cranial vault were involved, although the involvement was principally in the frontal and parietal bones.

Histologic examination of elective bones was carried out in all 6 cases. Examination of paraffin sections stained with hematoxylin and eosin was the standard method.

The bone on the edges of the lacunas was thrown up into ridges which completely surrounded the lacunar bases. This "ridge reactive" bone was lamellar in type, with well demarcated cement lines and the beginning of haversian systems. There was no zonal osteoclasia. There was a sharp line of transition between this lamellar-cement line bone and the tissue filling the defect (fig. 3).

The tissue filling the defect showed variability of type. In some regions the defects were filled in with feeble droppings of osteoid with parallel lines of osteoblasts or with active osteoblasts and endosteal tissue, with feeble markings of woven bone. Other regions showed dropping out of osteocytes, crumbling and fragmentation of osteoid material, or loose endosteal tissue, capillarized beds and shadows of decadent osteoblasts outlining lightly stained osteoid (fig. 4).

In an occasional focus of lacunar tissue, the inner table was represented by a vascular zone of large capillaries, filled with erthrocytes. A number of the defects were made up of a thin strip of fibrous tissue with complete absence of bone formation or with regions of failure of fibroblasts to transform into osteoblasts.

Examination of the clavicles and other bones showed the osteoid seams of standard width. There was no evidence of a specific inflammatory lesion. There was a balance between osteoid and calcification. This is good evidence against syphilis being the causal agent in this anomaly-complex. The absence of osteoclasia in the ridge defect zone is histologic evidence against the pressure theory.

Morphologic examination of the sections, we felt, was in favor of complete or partial failure of fibroblasts to transform into osteoblasts, with ensuing poor production of osteoid and feeble ossification.

---

19. Hartley, J. B., and Burnett, C. W. F.: J. Obst. & Gynaec. Brit. Emp. **50**:1, 1943.

The recognition of this condition in stillborn infants is against rickets or scurvy playing a role in the development of this defect. The fact that in the sixth case the mother was exposed to measles hints at the possibility of this infectious process being incriminated.

THEORIES OF ORIGIN

The cause is unknown. Three theories have been proposed, none of which has been proved.

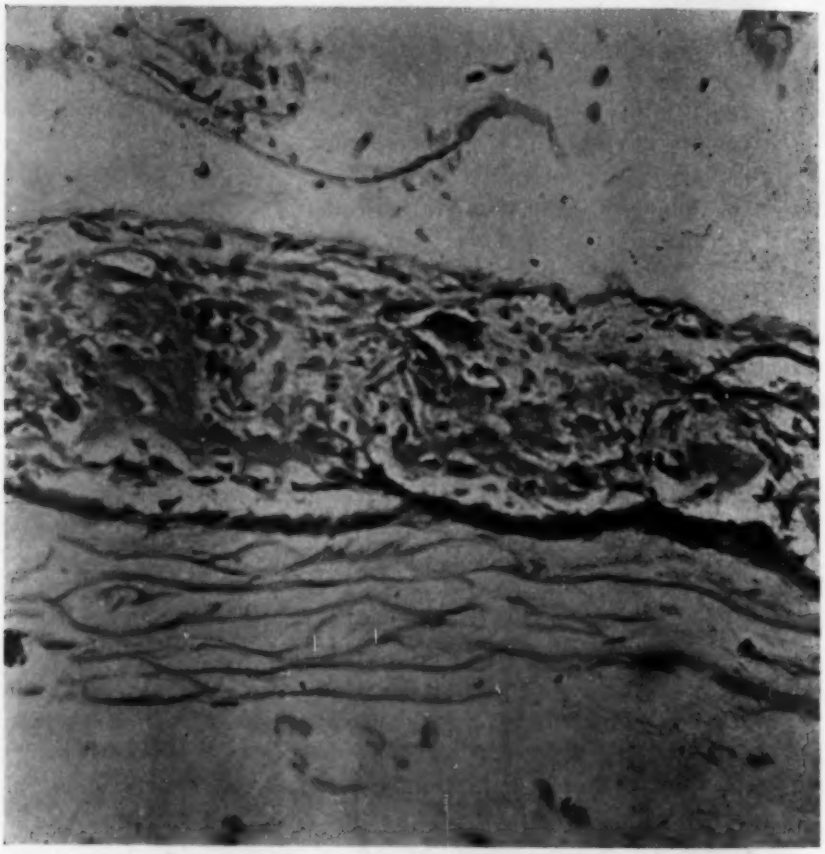

Fig. 4.—Tissue from a defect, showing woven osteoid; x 450.

1. *Intrauterine Pressure Disturbance.*—According to Faust[20] there is in intrauterine life a physical balance between the intracranial pressure (e.g., the pressure of cerebrospinal fluid and brain substance) and the extracranial pressure (e.g., that of the amniotic fluid). During the middle and last months of fetal life, when the brain grows rapidly, a

20. Faust, H.: Beitr. z. path. Anat. u. z. allg. Path. **86**:613, 1931.

physiologic delay in the development of the skull occurs, which may result in appositional defects and softening along the margins of the sutures. A meningocele produces a disturbance in the normal hydrodynamics resulting in depletion of cerebrospinal fluids in the subarachnoid space owing to the reservoir action of the meningocele. This depletion allows a firmer contact between the brain and the skull, resulting in pressure atrophy. Engstler, forty years ago, put forward the theory that ischemia due to increased intrauterine pressure, rather than pressure atrophy, was the etiologic factor. This ischemia resulted in poor nutrition and impairment of normal bone development. Kassowitz'[21] theory of parturitional trauma has been disproved by the prenatal demonstration of the condition.

Wieland, in 1909, postulated that the increased pressure acting on the incompletely ossified portions of the skull interferes with its normal development. In his hands, histologic examination revealed thinning of bone in the defective areas, with normal new bone formation at the edges. This, Wieland felt, was indicative of resorption secondary to disturbed ossification, in turn secondary to mechanical pressure.

Schuller[22] stated that the condition was primarily one of deficient ossification, rendering the skull less resistant to abnormal pressure. Markoe stressed the fact that the defects do not correspond to the centers of ossification and therefore could not possibly represent deficiency of ossification.

According to Kerr, craniolacunia develops only during fetal life because after birth the excess intracranial pressure is dissipated in the meningeal sac. Yet Faust stated that dissipation of pressure is essential for the development of the defects.

This theory does not explain why the condition is not seen more frequently with hydrocephalus, or in some cases of oxycephaly, nor why it is not invariably associated with meningocele. It must also be pointed out that during fetal life the intracranial pressure is equal throughout, there being no localizing of increased tension. There is no appositional relationship between the lacunar defects and the convolutions of the brain. In addition, it is difficult to conceive the gelatinous brain of the fetus exerting pressure to produce lacunar depressions of the skull.

2. *Congenital Developmental Defects.*—The condition apparently falls into the same unsatisfactory category as the majority of congenital anomalies, the causes of which are still obscure.

---

21. Kassowitz, M.: Med. Jahrb. 1880, p. 315.

22. Schuller, A.: Roentgen Diagnosis of Diseases of the Head. St. Louis, C. V. Mosby Company, 1918, p. 39.

Hartley and Burnett felt that the condition was due to a developmental defect in which two processes are frequently associated, namely, faulty ossification of the primitive membranous vault which surrounds

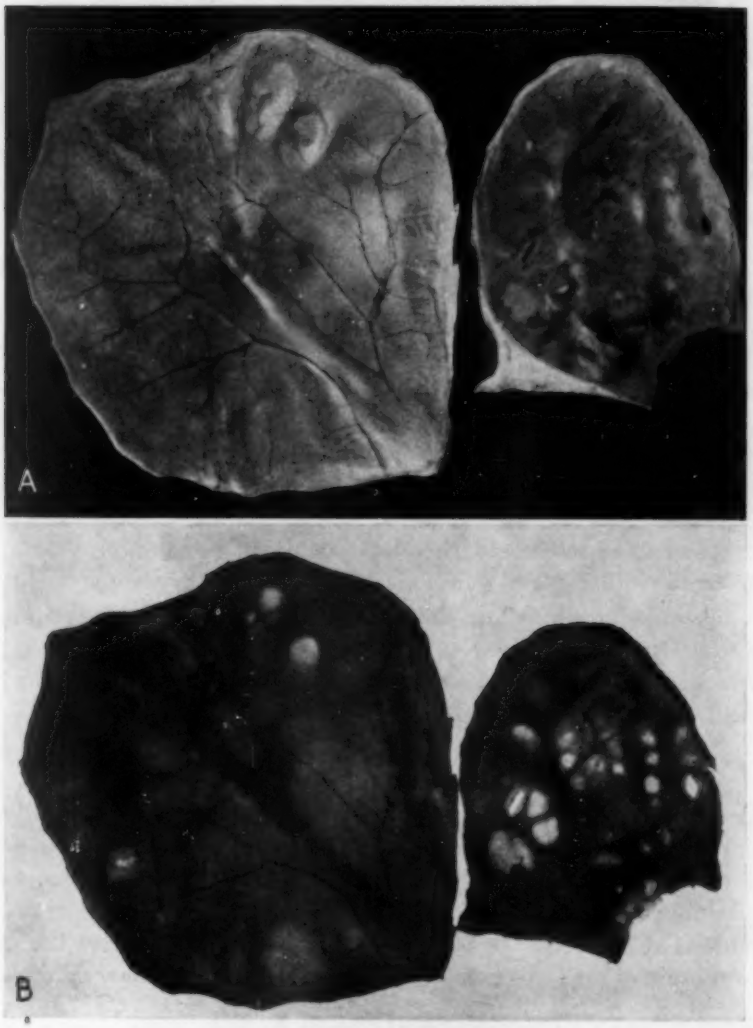

Fig. 5. (case 4).—*A*, photographs of the skull bones of the patient; *B*, transilluminated photograph of the same specimen.

the early brain, and faulty chrondrification of the vertebral bow of the primitive membranous spinal column, which surrounds the early spinal cord. This may be due to an inherent defect of a chromosomal nature in which dysplastic mesodermal tissue is produced. In case 4 there is

a suggestion of familial transmission. In case 6 it must be remembered that the mother was exposed to measles during pregnancy, as the rubella virus has been labeled as an agent producing congenital stigmas.

3. *Causal Diseases.*—Hartley and Burnett, in 1944, changed their views and were inclined to base the causation on a nutritional deficiency. Hughes thought that a nutritional or an organic disease such as rickets or syphilis was responsible for craniolacunia. Hartley and Burnett[23] did not find that it was constantly related to rickets, syphilis or toxemia of pregnancy, nor did they find the presence of any causative abnormal pressure effect either within or without the skull, before or after birth. In most cases the Wassermann test shows no reaction for syphilis, and there are no other indications of syphilis and no signs of rickets. Syphilis may produce osteitis or even perforations of the skull, but the roentgenologic and histologic pictures are different.

In the 6 cases included here there was a negative reaction of the maternal serum for syphilis. The stillborn and neonatal infants examined were free of the stigmas of syphilis.

COMMENT

The diagnosis of craniolacunia is made either during life by roentgenographic studies or at autopsy by direct inspection, transillumination or by use of postmortem roentgenography (fig. 5).

The skull, on postmortem examination, may show widened sutures or fontanels and may feel plastic. Further investigation reveals that the areas of rarefaction, the lacunas, give rise to an arborizing network of ridges which is diagnostic. The presence of brain and cord defects should evoke closer examination of the skull to demonstrate craniolacunia.

The pathologic investigation of stillbirths and neonatal deaths tends to be cursory. Reports of the morbid anatomy of lacunar skull and associated anomalies are scanty. The stress previously has been from a pediatric and roentgenologic standpoint. A more detailed autopsy study of this group may possibly reveal a greater incidence of neuroembryologic defects and shed further light on the histologic aspects and the causation.

SUMMARY

Six cases of craniolacunia are described, with the pathologic observations. The incidence in a general hospital is noted. Theories of origin are discussed, and support for one is offered. The attention of the general pathologist is drawn to these neuroembryologic defects as a source of material requiring investigation.

---

23. Hartley, J. B., and Burnett, C. W. F.: Brit. J. Radiol. **16**:99, 1943; Arch. Dis. Childhood. **18**:173, 1943.

Notes and News

Appointments, Etc.—H. N. Marvin, of the University of Arkansas School of Medicine, has been appointed head of the department of biology of the M. D. Anderson Hospital for Cancer Research, Houston, Texas. Dr. Marvin will be engaged in research on the relation of hormones to cancer and growth in general.

F. William Sunderman, professor of clinical pathology and director of the laboratory of clinical medicine at Temple University Medical School, has been appointed head of the department of clinical pathology at the Cleveland Clinic Foundation.

H. C. Sweaney, director of research at the Municipal Tuberculosis Sanitarium, Chicago, has been given the Dearholt Medal, awarded annually by the Mississippi Conference on Tuberculosis for outstanding work on the control of tuberculosis.

P. A. Herbut, assitant professor of pathology, Jefferson Medical College of Philadelphia, has been made professor of pathology to succeed Virgil H. Moon, who is retiring at the end of this year.

Carl E. Duffy has been appointed head of the department of bacteriology and parasitology of the University of Arkansas, Little Rock. Dr. Duffy was associate professor of bacteriology at Wayne University, Detroit. During the war he conducted research work on Japanese encephalitis virus at the Rockefeller Institute.

Deaths.—Louis Berger, professor of pathology at Laval University, died in Quebec, Canada, August 31, last, 52 years old.

Society News.—The 1948 annual scientific meeting of the American Society for the Study of Arteriosclerosis was held October 31 to November 1 at the Hotel Knickerbocker in Chicago.

Legal Medicine.—The Medical College of Virginia, Richmond, will inaugurate a department of legal medicine, under the direction of H. S. Breyfogle, chief medical examiner of Virginia.

The National Heart Institute.—The program of the new institute, established in the United States Public Health Service, will include research, financial aid to institutions for research and training of professional personnel, provision of fellowships to individual scientists, and grants-in-aid and technical assistance to the states for the development of heart disease control services.

Conference on Coccidiomycosis.—A research conference is planned by the New York Academy of Sciences for March 3 to 4, 1949, at the American Museum of Natural History in New York, and investigators in this field throughout the country will be invited to attend. The organizing committee is anxious to receive suggestions that will aid in making the conference an extremely successful one. Further details may be obtained from Sterling Brackett, American Cyanamid Company, 1937 West Main Street, Stamford, Conn.

Laboratorio de Investigaciones Histologicas "Del Rio Hortega," Buenos Aires.—This laboratory which carries the name of its founder, the late Spanish scientist P. del Rio Hortega, is now located at Montevideo 81, Buenos Aires, Argentina. Under the patronage of the Fundacion Roux, the continuation of the work of its founder on histology and histopathology is directed by M. Polak.

Books Received

LES FACTEURS CHIMIQUES DE CANCÉRISATION: LE PROBLÈME DES SUBSTANCES CANCÉRIGÈNES ENDOGENES. By René Truhaut, docteur en pharmacie d'état, chef du service de chimie à l'Institut du Cancer de l'Université de Paris, pharmacien-chef des Hôpitaux Psychiàtriques de la Seine, lauréat de la Faculté de Pharmacie de Paris. Paper. Pp. 173, with 6 illustrations. Paris (Ve), France: Société d'Édition d'Enseignement Supérieur, Siège social; 99, Boulevard Saint-Michel, 1947.

This monograph presents a summary of experiments conducted by the author since 1930, correlated with reviews of the literature. It is divided into four parts which deal respectively with (1) chemical carcinogens and theories of their mechanism of action, (2) numerous experiments with 3,4-benzpyrene, using five species of animals, (3) the carcinogenic action of certain alimentary substances and (4) the problem of endogenous carcinogenic substances. Data are given on many subjects not apparent from this outline. These include, among others, improvements in the synthesis of 3,4-benzpyrene, metabolic products of 3,4-benzpyrene, attempts (unsuccessful) to produce cancer in tissue culture, the role of glutathione in carcinogenesis, the absence of the carcinogenicity in wheat germ oil, and the failure to produce carcinogens from cholesterol with roentgen radiation. The chief emphasis is on attempts to isolate and identify the carcinogenic substances occurring in human tissues and blood. These tissue carcinogens were successfully concentrated but not isolated. There is a valuable bibliography with 421 references. This is an admirably critical monograph which should be available to all investigators in the special fields mentioned and to those interested in the etiologic aspects of cancer.

ESSENTIALS OF PATHOLOGY. By Lawrence W. Smith, M.D., F.C.A.P., formerly professor of pathology, Temple University School of Medicine, and Edwin S. Gault, M.D., F.C.A.P., associate professor of pathology and bacteriology, Temple University School of Medicine. With a foreword by the late James Ewing, M.D., Memorial Hospital, New York. Third edition. Pp. 764, with 740 illustrations. Price $12. Philadelphia: The Blakiston Company, 1948.

This book, first published in 1938, is a notable pioneer effort to teach gross and microscopic pathology and its clinical application by the case history method. The text has been revised and condensed. New material and new illustrations have been introduced without increasing the size of the book. General and special pathology are well presented and applied on the basis of 261 case reports, with 740 figures, mainly black and white and nearly all original. The book will be of interest in the teaching and study of pathology.

A-B-C's OF SULFONAMIDE AND ANTIBIOTIC THERAPY. By Perrin H. Long, M.D., F.R.C.P., professor of preventive medicine, Johns Hopkins University School of Medicine; physician, Johns Hopkins Hospital. Pp. 231. Price $3.50. Philadelphia and London: W. B. Saunders Company, 1948.

A highly informative book for practitioners, based on the author's use of sulfonamides and antibiotics, which covers some 12 years. It provides reliable guidance for those who treat infectious diseases with these drugs, of which only the ones of proved value are considered.

METHODS IN MEDICAL RESEARCH: VOLUME I. Van R. Potter, editor in chief. Assay of Antibiotics, Henry Welch, editor; Circulation—Blood Flow Measurement, Harold D. Green, editor; Selected Methods in Gastroenterologic Research, A. C. Ivy, editor; Cellular Respiration, Van R. Potter, editor. Pp. 372, illustrated. Price $8. Chicago: The Year Book Publishers, Inc., 1948.

This volume is the first of a projected series to be published annually under the direction of a distinguished governing board. It contains selected methods and technics in four fields of research, namely, essay of antibiotics, circulation—blood flow measurement, selected methods in gastroenterologic research, and cellular respiration. These four sections are edited respectively by Henry Welch, Harold D. Green, A. C. Ivy, and Van R. Potter. Each section in turn has many contributors and reviewers. It is planned that succeeding volumes will give research methods in other fields of medicine and ancillary sciences. The book is not suitable for detailed critical review. The sections vary greatly in quantity and in completeness. Some technics are presented without comment concerning their use or limitations; others are accompanied with brief discussions. In the opinion of the reviewer, subsequent volumes should conform to the latter plan. Research technics are constantly undergoing improvement, and the governing board has undertaken a big task, but a worthy one if in publishing these volumes it also keeps them up to date.

RETICULOSIS AND RETICULOSARCOMATOSIS: A CLINICAL AND PATHOLOGICAL STUDY. By Dr. P. Van Der Meer and Dr. J. Zeldenrust, of the Medical Clinic of the University Hospital, Leyden, Holland, and the Pathological Laboratory of the State University, Leyden, Holland. Cloth. Pp. 83, with 2 tables and 21 figures. Leiden, Holland: Universitaire Pers Leiden, 1948.

This short monograph is based on a detailed study of 7 cases of aleukemic reticulosis and 11 of reticulosarcoma. Reticulosis is regarded as a neoplastic rather than as a hyperplastic increase of reticulum cells. It differs from reticulosarcoma in its diffuse systemic nature with no localized tumors, although transition forms occur. The pathologic aspects, the clinical picture, the differential diagnosis and the treatment are briefly mentioned. Twenty-one photographs and photomicrographs illustrate many points under discussion. Perhaps the most valuable contribution of this monograph consists in its tracing of the evolution of cencepts regarding these two diseases. Many of the old controversies on classification and etiologic factors appear outmoded in the light of recent experimental work.